

Is world government on the cards?

Last weekend's meeting at Naples suggests that the world's statesmen are coming to the view that stronger institutions of world government are now an urgent need. Not before time.

THE notion that there might at some stage be a unified government of the whole world is usually, and rightly, regarded as a wild dream. With all the squabbling about us, from North Korea's nuclear bomb-rattling to Europe's recent failure to agree on a successor to M. Jacques Delors as president of the European Commission, and not to mention Bosnia and Herzegovina, is it not idealistic nonsense to nourish the pan-federalist dream? That is the conventional put-down. Curiously, that seems partly and unexpectedly to have been undermined by the meeting of the political heads of seven industrialized countries at Naples last weekend (with President Boris Yeltsin joining for the second half). Idealists everywhere may now cheer up.

But none of that implies that political heads have abandoned self-interest for idealism. Rather, self-interest impelled them to the view that something must be done to strengthen existing international institutions, organizations such as the International Monetary Fund (IMF), the World Bank and the International Bank for Reconstruction and Development. Indeed, even the United Nations (UN) is ripe for reconsideration; the five permanent members of the Security Council (each of which can veto any resolution agreed by the others) are Britain, China (which replaced Taiwan a decade ago), France, Russia (which seamlessly inherited the mantle of the Soviet Union) and the United States. Some of this (but probably not all of it) is to be argued out at the next meeting of the G7/G8 summit this time next year, at Halifax, Nova Scotia.

Why all the hurry, and so suddenly? For one thing, the Cold War is over for all practical purposes. Indeed, the well-timed death of Kim Il-Sung, the president of North Korea, on Friday last week may yet mean that the Cold War is finally at an end. (The negotiations due to begin in Geneva after Kim's funeral should make it possible to tell.) But what used to be called the 'peace dividend' has disappointed the West and even Japan. The buoyant prosperity of the later stages of the Cold War has not reappeared, but East Asia is booming away, promising a decisive shift of economic and ultimately of political strength. But the past few years have seen a string of sour disappointments in the capacity of the world's most powerful governments to bring civility to places such as Iraq, Somalia, Haiti, the Caucasus and surrounding regions, the republics of what used to be Yugoslavia, and Northern Ireland. No wonder they are bewildered by the pace of change, and that they seek greater assurance in a more robust set of international institutions.

The danger is that this time next year is several years too soon. The difficulty is not that of specifying mechanisms for abating the tensions generated by economic growth and rootless barbarism, but of getting governments at large to agree to any one of them. (It was much easier when the governments of the still-free world met at San Francisco in 1945 to create the United Nations.) Moreover, general agreement on any single ordering framework is unthinkable unless derived from a series of regional understandings. Yet the European Union (EU), the best simulation yet of a coherent regional grouping, plans only for 1996 a conference to discuss a framework within which unified external policies might be moulded (and that will not be easily done).

So the idealists will have to be patient for a little while, but that does not mean that all is lost. President Bill Clinton was on the right track when he urged at Naples a speedy attempt to build on the agreement under the General Agreement on Tariffs and Trade (GATT), reached with difficulty at the end of last year. (And President François Mitterrand was right to protest that it would be better to get that agreement ratified before moving further.) The virtue of that approach is that it should be possible to broaden the membership (by including China particularly). Reform of the IMF's procedures for helping members in financial trouble is overdue, but so much is agreed. But the experience of the past three decades has shown that there is nothing like world trade to cement even disparate countries together by the stratagem of interdependence.

But more than mere trade is necessary. As the often abortive intervention by UN forces has shown in the past few years, trade alone will not still hostilities between people sharing a common territory. And while any sovereign state can belong to the UN, members' obligations are merely to maintain international peace and security, and to conduct

One-day conferences

The Berlin meeting, *Genetics in Europe now*, will be held on Friday 30 September 1994 at the Brandenburg Akademie, and the cost is DM195 or £75 (plus VAT).

The Paris meeting, *What is distinctive about European science?*, will be held on Wednesday 26 October 1994 at the Ecole Normale Supérieure. The cost is FF675 or £75 (plus VAT).

Payment may be made by cheque (payable to Macmillan Subscriptions Ltd) or credit card. For further details of either meeting, or to reserve places, please write, telephone or fax to Pippa Burnett, Nature Conferences Office, 4 Little Essex Street, London WC2R 3LF, UK; telephone +44 71 836 6633 ext. 2593; fax +44 71 379 5417.



their domestic policies in ways conducive to those ends. Moreover, while pariah states can in principle be expelled, that is not much of a sanction. The obligations of membership need to be made more stringent. Yet it is plain that not all members of the UN follow the second precept. Perhaps the place to start is for the Security Council to take some of the worst offenders to the International Court of Justice. That would help to accumulate case law that differentiates the acceptable from the unacceptable. It would do no harm if the idealists began saying that. □

Research for money

The latest of the British government's plans for research are mercifully only proposals at this stage.

THE upheaval within the British research establishment continues, now with the proposals for reorganizing public research laboratories engaged in civil (as distinct from military) research. The first thing to be said about the proposals (see page 89) is that the government has wisely allowed four months (until 11 November) for interested parties to have their say; let us hope that consultation means what it should. The second is that, whatever decisions are taken later in the year, it will be virtually impossible for a British research council or government department to create a new free-standing laboratory; those that will not be privatized or amalgamated when the dust has settled will mostly be working on commercial terms for identifiable customers, many of whom will be government departments. The third is that British universities could be the chief beneficiaries of the upheaval if they play their cards right.

The new proposals, in whose preparation the government's Efficiency Unit has played a leading part, amount to the apotheosis of the Rothschild proposals of 1971. Every project will have its commissioning customer and its price. That is one clue to the way in which the proposals may go awry. Rothschild's proposals were enthusiastically endorsed by the Heath government of the day, but in the end had little consequence because the customers were found wanting. Government departments did not provide themselves with the chief scientists and their staffs who could accurately identify their own departments' needs, let alone write meaningful contracts with the laboratories from which they commissioned research. The most curious feature of this week's report is that it says nothing on that score. Have things much changed since 1971? Or will the government departments, under budgetary pressure, decide that, because they do not understand how research could help them, they need commission none? This is part of what happened last time.

The particular proposals are in many ways quaintly ideological. Take one example: the National Radiological Protection Board (NRPB) is sited (in Oxfordshire) alongside the Radiobiology Unit of the Medical Research Council (MRC). The former has statutory functions in the definition of allowable exposure of people to radiation and the latter a research interest in the effects of radiation on living tissues.

It is true, as the report notes, that the two establishments are "geographically close and have overlapping interests", but they have different goals. So why should MRC and the Health Departments "consider bringing together" part of the unit with NRPB? Is that more than a ritual declaration that amalgamation means efficiency?

The animus against 'overlap' shines through the government's proposals, but often imprudently. Take, as another example, the British government's present difficulties over bovine spongiform encephalopathy (BSE), where most current research is done at the Central Veterinary Laboratory (CVL). Without casting doubt in any way on the quality of the work, it would be reasonable that an alert chief scientist would wish to commission research with the same goals at a second centre, at the very least. To some extent this happens, but prion diseases are not traditionally at the centre of concern of the veterinary laboratories. Is that not a warning that there are many circumstances in which an alert government will welcome the diversity which its efficiency unit seems determined to destroy?

How may British universities benefit from all this? By bidding for laboratories on the privatization list. The efficiency review suggests that the virus laboratory at Oxford, now a dependant of the Natural Environment Research Council, should be transferred to a neighbouring university. No doubt there will be many university heads going through the detailed proposals in the next few weeks in case there is a nearby laboratory for which they can bid. In the short run, they will be able to make satisfactory deals with a government apparently eager to shed its research arm. The rub would come only when the time came to apply for research grants to continue the research, by which time the original customers might have walked away. That is a risk worth taking. □

Nature's birthday party

Will readers please declare their interest in the two one-day meetings arranged for later in the year?

ARRANGEMENTS for *Nature's* celebrations of its 125th anniversary on 4 November are now almost complete. Several projects are under way, many of them taking the form of conferences. That seems especially worthwhile, given that the editorial staff of a busy journal is less able than it might be to meet the members of the club of readers and contributors that any journal of this kind must be. But the topics chosen are interesting and important in themselves.

The first one-day conference, at Berlin at the end of September, will deal with the application of genetics in biotechnology and the genetics of disease. The hope is that the result will be a better understanding of European policies on the regulations of genetics, in its research and application. The second meeting is in Paris a month later, on 26 October, and will deal with the distinctiveness of European science. It would be extremely helpful if those who plan to attend could signal their intentions at an early stage. Details appear on the first page of this issue. □

France wins larger share of patent royalties after AIDS test dispute

Washington. The Pasteur Institute in France has won a three-year battle with the US National Institutes of Health (NIH) for a larger share of patent royalties from AIDS tests developed jointly by scientists at the two institutions.

Under a deal agreed on Monday by the French and American AIDS Foundation (FAAF) — the body established in 1987 to distribute the royalties — the NIH formally acknowledged that the test kit that it developed made use of a virus provided by the Pasteur Institute.

In future, FAAF will distribute the patent royalties according to a new formula that will give the Pasteur Institute more money than the NIH. But Harold Varmus, director of NIH, says that the new deal is consistent with the intention of the original 1987 agreement, as it gives both countries equal shares of the royalties over the full life of the patent.

Up to now, the United States has been receiving a larger share of royalties than



Burying the hatchet: Varmus of NIH (left) and Schwartz of the Pasteur Institute.

France — so far it has received \$20 million and France \$14 million — as the US AIDS test kit has had far larger sales than the French one.

The United States is seeking to characterize the new deal as an adjustment needed to address this apparent anomaly. But the French are presenting it as a victory on an important matter of principle. Either way, the resultant compromise should at least

restore working relations between Pasteur and NIH.

Under the 1987 agreement, both countries keep 20 per cent of their kit royalties, and pool the remaining 80 per cent. The old agreement gave one quarter of the pooled money to the World AIDS Foundation (WAF), which funds AIDS research and education in developing countries, and divided the rest equally between the US and France. The new deal gives France half of the total, and the United States and the WAF one quarter each.

According to both parties, the deal resolves the argument between Pasteur and NIH on the matter for good. But it will not resolve the question of whether the NIH research team, led by Robert Gallo, misappropriated the French virus accidentally or deliberately.

Gallo attended the FAAF meeting and endorsed the deal. In a statement issued through his lawyer after the meeting, he claimed that the deal “simply implements the spirit of the 1987 settlement”.

Pasteur had originally sought an agreement that would have given it three-quarters of the pooled money, the WAF a quarter, and the NIH nothing. But director-general Maxime Schwartz says that his earlier stance was merely a negotiating position, and that he is satisfied with the final outcome.

“I’m very pleased that a decision was taken to change the distribution of royalties in a way that is much more fair than it was before,” Schwartz said after the meeting, adding that the formal acknowledgement from the US side that the US test was based on the French virus was more important to Pasteur than the financial settlement.

Asked why he had changed his mind between 8 June, when he rejected Schwartz’s pleas for a renegotiation of the deal, and 23 June, when he offered talks (see *Nature* 369, 693; 1994), Varmus said that conversations with Schwartz during this period had made him see the dispute “in a slightly different context”.

Varmus said the appearance of a report prepared by the inspector general of the health department on 10 June had “very little” impact on his change of mind. He says that the decision to negotiate was his alone, and denies that it had been influenced by pressure from the chairman of the House energy and commerce committee John Dingell (Democrat, Michigan), whose staff have kept a keen interest in the Gallo case. Varmus adds that he sees no need for the Office of Research Integrity, which last year dropped a case against Gallo, to revisit the matter.

Colin Macilwain

Bomb builders ‘motivated by fear’

Moscow. Russian scientists have claimed that the Soviet physicists involved in building an atomic bomb in the late 1940s were motivated by the fear that, if they failed, they would be dismissed as ‘idealists’ and suffer the same fate as biologists had done under Joseph Stalin.

In an open letter, the scientists admit that the construction of the first bomb, which was successfully tested in 1949, was made possible only because secrets were transferred from the West (in contrast to two further atomic weapons tested in 1951 — as well as the later hydrogen bombs — that were products of Russian science).

They also criticize recent claims made in the book *Special Tasks*, written by the former Soviet spy, Pavel Sudoplatov, that the physicist Robert Oppenheimer was involved in passing nuclear secrets to the Russians. (Last week, the son of Lavrenti Berio, who was responsible for the security surrounding the Russian bomb project, added further speculation to this story by claiming that Oppenheimer had stayed at his father’s house in 1939.)

The signatories to the letter, headed by Vitaly Goldansky, until recently the head of the Chemico-Physical Institute of the Russian Academy of Sciences, express particular concern that history is being ‘rewritten’ in a way that

presents their immediate predecessors as “mere imitators” of work carried out elsewhere, based on blueprints obtained by Soviet spies operating in the West.

Voicing their support for a leading article in *Nature* (368, 779; 1994) which condemned the inferences in the book that not only Oppenheimer but also Niels Bohr, Enrico Fermi and Leo Szilard were involved in passing nuclear secrets to Russia, they warn of a growing tendency to make science a scapegoat for Russia’s current difficulties.

But, in what is perhaps the most intriguing suggestion, they argue that physicists in the 1940s faced the prospect that those who expressed belief in the theory of relativity would be condemned as ‘idealists’ — and would therefore suffer the same fate as those who opposed the ideas of ‘man-directed’ evolution propagated by Stalin’s protégé, Trofim Lysenko.

The successful demonstration of nuclear weapons apparently put an end to this threat, and physicists subsequently came to occupy positions of power and prestige in the post-war Soviet Union. Signatories to the open letter in addition to Goldansky include Valery Subbotin, Alexander Chudakov, Alexander Fokin, Mikhail Styrikovich, Yuri Osipian, Zhores Alferov, Alexander Skrinisky, and Alexander Andreev. Carl Levitin

Japan bids to form new centres of excellence

Tokyo. Japan's Science and Technology Agency (STA) will pump \$20 million of extra funds over the next five years into each of two national research institutes in the hope of converting them into 'centres of excellence' (COEs) able to compete with the world's leading research institutions.

The two institutes, whose names were announced last week, are the National Institute of Agrobiological Resources of the Ministry of Agriculture, Forestry and Fisheries, and the Communications Research Laboratory of the Ministry of Posts and Telecommunications.

They join three other institutes chosen as potential centres of excellence last year (see *Nature* 364, 660; 1993). But critics say it will take a lot more than just money to bring about the change.

Japan's centre-of-excellence scheme is unique among such government-funded schemes around the world in that money is directed not at institutes already judged to conduct research of a high standard, but rather those judged capable of doing so in the future. One result of this policy is that some of Japan's best research institutes stand little chance of getting an award.

In theory, more than a hundred national research institutes and semi-government research organizations affiliated to Japan's

various ministries and agencies could apply for COE funding (those affiliated to the Ministry of Education, Science and Culture are excluded from consideration).

In practice, however, comparatively few institutes have officially applied. This year, for example, only 12 institutes did so, compared with 19 in the first year of operation of the scheme. The two selected were chosen after they had been screened by a committee of 17 leading academics and industrialists, headed by Wataru Mori of the Council of Science and Technology, Japan's leading policy-making science body.

Most institutes are eliminated at an earlier stage by unofficial internal screening within the various ministries and agencies. For example, Japan's well-known Institute of Physical and Chemical Research (RIKEN) was hoping to apply this year.

RIKEN officials had planned to seek funds to reinforce some of its weaker divisions. But they were informally told by STA that this idea did not fit its strategy of supporting institutes as a whole, and subsequently decided not to proceed with their application.

Similarly, the National Cancer Center Research Institute has held back from applying for COE funds because it is in the middle of negotiations for funds for a new

10 year cancer programme.

STA officials insist that external factors do not influence the selection of institutes for COE funding, and that any institute can apply. But they do admit that each ministry and agency probably decides through its own internal processes which of its institutes it will put forward for COE funding.

A researcher at one of the five institutes already selected says he is sceptical that the COE funds will achieve their goal of nurturing excellence. Extra money helps, he admits, but will not in itself address fundamental problems in the management and administration of many national institutes.

He says the heads of institutes are often out of touch with the latest developments in science. And, with the exception of a few institutes, such as RIKEN, there is no process of external review of institutes. As a result, most researchers are not doing first-rate research, yet they are in a position to influence the decision-making of the institute head and its management.

A review of each of the COE-funded institutes will be carried out in the third year of their five-year term of funding by the committee that selected the institutes. But no external review by scientists unrelated to the programme is planned.

David Swinbanks

Citation analysis confirms Australian science's declining influence

Sydney. The quality of science in Australia relative to that elsewhere seems to have fallen markedly since the mid-1980s, according to a detailed analysis of the number and citation rate of papers produced by Australian scientists and published last week.

Paul Bourke and Linda Butler, of the Performance Indicators Project at the Australian National University's Research School of Social Sciences, found that Australians still produce about the same proportion of the world's research papers — about two per cent — as in 1987.

But, building on a theme first raised last year by the US Institute for Scientific Information (ISI) in its publication *Science Watch* — and following similar work on British science carried out at the University of Sussex's Science Policy Research Unit — they also found that the citation rate had fallen by 25 per cent over the same period.

The breakdown of publication and citation rates by disciplines in the analysis of ISI data reveals some large variations.

For example, Australia has managed to maintain its long-standing prominence in agriculture and earth sciences. Similarly, the citation rate remained reasonably stable in medical science, industrial biotechnology and food sciences.

But citation rates in the non-medical

biological sciences, in physics and in chemistry have each fallen below the international levels they had reached in the mid-1980s. Even more worrying, citation rates dropped by 41 per cent in information, computing and communication technologies.

A comparison of the results with the performance of other countries in the ISI analysis showed that only one country (Sweden) has a similar pattern of producing as much research as a decade ago but being quoted less widely.

Canada, Germany, Japan, the Netherlands, China and South Korea are each producing more research, and this is being cited more often. Researchers in France and Switzerland are contributing a stable proportion

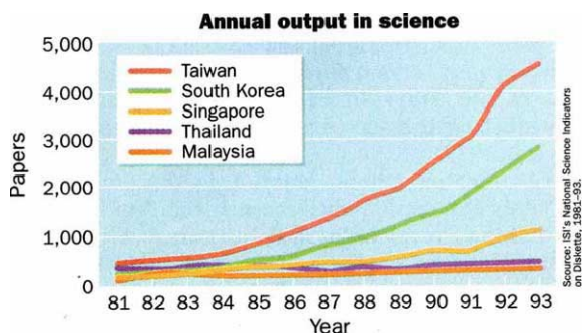
of papers, but they are earning more citations.

Bourke points out that countries in which citation rates have been declining — namely Australia, Sweden and Britain — are also those in which a relatively high proportion of research is performed in universities. But he emphasizes that a lot of work is still needed before the reasons for the decline can be properly identified.

He speculates that possible reasons could include a general shift from basic to applied research — a major theme emphasized in government funding for science — the ageing of the Australian scientific community and a decaying research infrastructure.

Mark Lawson

While Australia reflects on the declining impact of its science output, figures just released by the ISI (right) reveal that its Pacific Rim neighbours are enjoying a steady increase in the number of papers published and cited. Singapore in particular has seen a surge in papers published from 167 papers in 1981 to 1,220 in 1993.



US universities fear fall-out from funding row

Washington. Leading US research universities are growing concerned that what initially appeared to be a relatively minor spat in Congress over defence research spending could turn ugly. The results could expose weakening political support for the physical sciences in the aftermath of the Cold War.

The dispute originated in a proposal last month by the House of Representatives' defence appropriations subcommittee, chaired by John Murtha (Democrat, Pennsylvania), to halve the \$1.8 billion allocated to the Department of Defense (DoD) for research in universities (*Nature* 369, 694; 1994).

The move is widely seen as a negotiating ploy. When the House approved the measure on 29 June George Brown, chairman of the Science Committee, expressed optimism that the problem will disappear in the Senate — and in the subsequent conference with the House. "There is no fight over this cut because, for all practical purposes, there is

no cut to fight over," said Brown. But members have told university officials that the earmarking issue is not the reason for the threatened \$900-million cut. Rather, it is apparently intended to persuade the DoD to select one of six major pending weapon systems for elimination.

The multi-billion dollar systems include new fighters for the Air Force, submarines for the Navy and helicopters for the Army. Murtha is effectively telling the Clinton administration that, unless it can find the courage to cut one of these systems, universities will suffer.

The institutions that would bear the brunt of the cuts, such as the Massachusetts Institute of Technology (MIT), Stanford University and the California Institute of Technology (Caltech), are relying on their friends in the Senate to get them off the hook.

But the Senate defence appropriations subcommittee has never been particularly friendly to such institutions. Many of its members come from states such as South Carolina and Montana whose voters have little sympathy for the plight of elite institutions in California and on the east coast.

University lobbyists in Washington are confident that they can get the \$900-million cut reduced to \$100 million or less. But some in the universities are less optimistic. They are concerned that no one outside the

universities has rallied to their cause; the computer and aerospace industries, for example, have been ominously silent.

Their silence is curious, as military-funded research in universities directly underpins those industries to an extent that is rarely admitted. Across the US university system, for example, the DoD funds 55 per cent of computer science research, 70 per cent of advanced materials research, and 80 per cent of electrical engineering research.

"People don't understand that, in terms of the long-term development of dual-use technologies, the DoD has been the visionary of the federal government," says Ken Campbell of MIT, which received \$70 million from DoD last year. Campbell points out that university research funded by the Defense Advanced Research Projects Agency (DARPA, now known as just ARPA) virtually created the US computer industry, and is central to the Clinton administration's information superhighway.

During the Cold War, it suited all parties to accept the DoD's role in supporting the elite US engineering schools and fulfilling the basic research needs of key industry sectors. Murtha's threat, intentionally or not, has put this role in the spotlight. Congress must now decide whether or not to continue the arrangement. **Colin Macilwain**



no cut to fight over," said Brown.

But some universities fear that such optimism is misplaced. "I'm very worried that people have been lulled into a false sense of security because the cut seems so large that it [appears] unlikely to happen," says Dick Zare, a chemistry professor at Stanford University, California, who is one of those fighting the proposal. He says the rationale for the proposed cut goes far beyond the disagreement between Murtha and Brown over Brown's efforts to reduce 'earmarking' of funds for pet projects, a reason widely quoted as motivating Murtha's committee.

Murtha has publicly justified the proposed cuts by claiming that universities are wasteful and ungrateful for DoD support. He has threatened to hold hearings on indirect costs — the universities' least favourite subject. Zare says that both the size of the cuts and the rhetoric that has accompanied them should warn the universities of the seriousness of the situation.

Brown's demands for an end to earmarking have certainly irritated Murtha's com-

NIH baffled by drop in applications

Washington. The US National Institutes of Health (NIH) has experienced a precipitous — but so far unexplained — fall in the number of research grant applications received from young investigators over the past nine years.

According to a report published in Washington last week by the National Academy of Sciences, the NIH awarded research grants in 1985 to 1,308 scientists under the age of 37. In contrast, the number of young investigators receiving their first NIH grant in 1993 was 527, a drop of 54 per cent. "We're puzzled," admitted Bruce Alberts, president of the academy, when asked where all the young scientists have disappeared to. "We don't know what's going on here."

The data revealing the decline emerged unexpectedly from a study conducted by the academy's National Research Council intended to assess whether young scientists have been disproportionately squeezed out of the system in comparison with senior investigators over the past five years or so, a period in which competition for limited NIH money became unusually intense.

In terms of what NIH calls the 'success rate' — the percentage of successful applicants — young scientists seemed to be holding their own. Shirley Tilghman of Princeton

University, who co-chaired the study with Torsten Wiesel of Rockefeller University, points out that although the success rate of young scientists declined slightly, the drop was not statistically significant.

Only when, late in the study, someone suggested looking at absolute numbers did the 54 per cent fall in young scientists getting NIH grants become apparent. Various explanations have been put forward, but so far each has been rejected as insufficient.

One possible explanation, for example, is that more women are entering the system, and moving up the academic ladder more slowly than their male counterparts. But the fact that women tend to be over 36 when they get their first independent grant does not account for the 54 per cent drop.

Given the uncertainties involved, the academy is now planning a follow-up study. This will both attempt to discover what has happened to the pool of young applicants for NIH grants, and will explore funding patterns at other agencies (and private foundations) that support research in the biomedical sciences. **Barbara J. Culliton**

The Funding of Young Investigators in the Biological and Biomedical Sciences. National Research Council. National Academy Press. 2101 Constitution Avenue N.W. Washington, D.C. 20418.

'Role models not important in helping women choose science'

London. Coinciding with the British government's decision to set up a 'development unit' to encourage more women to pursue careers in science, a survey of women scientists has shown that, contrary to popular belief, female role models are not a significant factor influencing such a decision.

The survey, carried out by the unit of policy research in science and medicine of the Wellcome Trust in London, is believed to be one of the first to back up anecdotal explanations on the paucity of women in science with hard data. Its conclusions are based on replies to questionnaires sent to 130 male and female undergraduates and postgraduates in physics and biochemistry at the Universities of Leeds and Cambridge.

Two-thirds of the women scientists questioned felt they did not need a role model to keep them in science, although most saw scientific research as a male-dominated area. The replies also revealed that, although 70 per cent of the women had decided between the ages of 11 and 16 to study science, a stimulating or challenging first degree course was a major factor in reinforcing such a decision.

The study's recommendations for making science more attractive to women coincide closely with those made by the Committee on Women in Science, Engineering and Technology in *The Rising Tide*, a report drawn up for the Office of Science and Technology (OST), and published earlier this year.

Specific proposals include the introduction of flexible working hours, with part-time work, job sharing and career breaks; help with the cost of child-care; schemes to help women taking 'career breaks' to keep

in touch; and the inclusion of more refresher training for those returning to work after a long break.

In an official government response to the committee's report, William Waldegrave, the minister of public service and science, told the House of Commons last week that the government would adopt the committee's main recommendation: it will set up a 'development unit' under the auspices of the OST, with two full-time members of staff, to oversee the promotion of women in science, engineering and technology.

The unit will initially run for two years, and will collaborate with other bodies to establish "women-friendly management practices" which it will encourage other public and private sector employers to adopt.

The unit will work with the Department of Employment to improve careers advice for women, and encourage employers to establish databases of women in science, engineering and technology. It will try to raise the profile of women scientists and their contribution in the media.

Jean Balfour, deputy chairman of the committee headed by Sir William Stewart, the government's chief scientific adviser, described the allocation of two members of staff as "significant" in the current economic climate. She also welcomed the setting up of the development unit as "a mechanism to move things forward".

But Balfour said the committee would be "disappointed" that recommendations on tax relief for child-care had been rejected. The government turned down this proposal because it is not prepared to make women scientists a special case. **Maggie Verrall**

Slovak minister lifts block on extra money for science

Bratislava. Slovakia's new government, formed after the fall of its predecessor in March, has agreed to top up research funds for this year with an additional SK110 million (around US\$3.5 million).

This sum had been promised by the last government, but only on condition that a new bill conferring legal status on Slovakia's



Harach: committed to boost science.

two grant-making agencies — one for basic research and one for applied research — was passed first (see *Nature* 368, 386; 1994). But Lubomír Harach, the new minister for education and science and former director of the ministry's Institute of Informa-

tion and Forecasting, has decided that this condition is no longer necessary.

Last month, Harach announced that nearly two-thirds of the money would go to applied research and just over a quarter to basic research. The remaining funds would be used to match money received for international collaborative projects.

Not everyone is happy. The Slovak Academy of Science argues that the Agency for Science should have a larger share of the money, as it has introduced a proper peer-review system for evaluating research proposals, in contrast to the Agency of Technology.

But Harach says that his allocation of the money is in line with the priorities he hopes to establish in Slovakia. He also says that he wants the government to address the need for a coherent long-term science policy.

Previous recommendations in this direction from the Organisation for Economic Cooperation and Development have been ignored by successive post-communist governments on the grounds that it should await development of a proper economic policy.

But Harach is already planning to put together an analysis of the current state of Slovakian research, and to compile a list of national scientific priorities. "This government gives great support to science and technology," he says.

Harach also plans to propose a separate ministry or state committee for higher education, science and technology, in order to give research a higher political profile and ensure that it is better coordinated. How much he will be able to achieve before the general election in September remains to be seen.

Allison Abbott

CNRS committee 'to break silence' on ethics

Paris. Europe's largest basic-research organization, the French Centre National de la Recherche Scientifique (CNRS), has launched an initiative to identify ethical problems raised by research in disciplines other than biology and medicine.

The new ethics committee, known as COMET, is the brainchild of François Kourilsky, the outgoing director-general of CNRS. Kourilsky has long argued that researchers must take account of the social acceptability of their work if they are to avoid a public backlash against it.

The development of nuclear technology, research on behaviour and the environment and the manipulation of historical research to political ends will all fall under the remit of the new committee, which will both produce expert opinions on topics submitted to it and take its own

initiatives. Another area to be reviewed will be problems associated with the dissemination of scientific results, and professional integrity.

The president of COMET is Hélène Ahrweiler, a historian, former rector of the Academy of Paris, and president of the University of Europe. She is determined that the committee will be more than a talking shop. Its main goal will be "to disturb, to break the silence", by asking "the right questions".

"Silence is the biggest enemy", Ahrweiler has said, claiming that the idea of "consensus" was invented so that "imbeciles wouldn't feel alone". Many important questions are never asked, she has said, claiming that the consequent vacuum is often exploited by "mafias and scientific pressure groups". Declan Butler

Privatization threat recedes for British labs

London. Two of Britain's largest research councils learned with relief this week that the government is not being advised by a team of 'efficiency' experts to require a major upheaval in their relationships with their research institutes.

In particular, neither the National Environment Research Council (NERC), nor the newly-formed Biotechnology and Biological Sciences Research Council (BBSRC), will be required either to sell off any institutes to the private sector, or even to deal with them at excessively arm's length.

At the same time, the Scottish Office has successfully argued that the government give close attention to it being allowed to take over responsibility for a wide range of government-sponsored research institutes based in Scotland.

Both conclusions have emerged from the eagerly awaited report of a team of civil servants set up at the end of last year under the government's 'efficiency adviser', Sir Peter Levene, to suggest ways of rationalizing 53 government research institutes.

Early fears had been raised that the group was intended to prepare the ground for rapid privatization. Indeed, one of its terms of reference was "to identify those public sector research establishments where early privatization is feasible and desirable".

But its report, on whose conclusions the government is inviting public comment partly in response to pressure from the scientific community, recommends that only one organization should fully take such a step, namely the Agricultural Development Advisory Service (ADAS) of the Ministry of Agriculture, Fisheries and Food (MAFF).

AIDS policy chief quits after criticism

Washington. President Bill Clinton's AIDS policy chief, Kristine Gebbie, has resigned after only 11 months in the job, and after mounting criticism of her performance.

Almost from the time of her appointment, AIDS experts said that the former Washington State health-care official lacked the political skills needed to fill the ill-defined 'AIDS czar' role that Clinton had promised to create during his election campaign.

No successor to Gebbie has been nominated. But names being circulated include that of Tim Westmoreland, a staff member on the House of Representatives health and environment subcommittee chaired by Henry Waxman (Democrat, California). AIDS activists and researchers are hoping that the White House will put some thought into redefining the job before it is filled. □

Similarly the report expresses little enthusiasm for an alternative idea, mooted by Levene himself in a separate report published last year, that all the institutions covered be linked into a single Civil Research Agency, comparable to the Defence Research Agency already set up to run the government defence research establishments.

In the end, the team has put forward two alternative solutions. One would be to link together government research institutes into four separate groupings each oriented towards a specific 'market sector'. These would cover the fields of marine resources and the environment; the non-marine environment; biotechnology and biological science; and food and agriculture.

Under such a solution — recommended in an preliminary version of the team's conclusions (see *Nature* 369, 5; 1994) — the four groupings would become the responsibility of the Scottish Office, the NERC, and BBSRC and the MAFF respectively.

Each grouping would have its own chief executive. But whereas the initial proposal requires four new appointments — a move which some feared could introduce a potential conflict within the two groupings run by research councils — the new proposal is that in these two cases, the role of CEO would be taken on by the CEO of the research council itself.

The alternative proposal put forward by the scrutiny team to create geographically-based groupings, in practice meaning a single grouping for Scotland, run by the Scottish Office, and three in England and Wales, run by the BBSRC, NERC and MAFF.

This proposal did not feature in the team's preliminary conclusions. Nor is it a solution favoured by the two research councils, which would each lose control of their institutes north of the border.

But it is one that has considerable political backing from the Scottish Office itself, keen to promote what it describes as the 'Scottish model' for organizing research — involving both the vertical and horizontal integration of research teams and institutes.

In addition to these two structural changes, the scrutiny team makes a number of general recommendations about how the effectiveness of government-funded laboratories could be increased. For example, it recommends that both government departments and the research councils "routinely examine" the potential for transferring the research institutes for which they are cur-

rently responsible to universities.

It also makes a number of specific recommendations for the future of institutions where it claims to have discovered overlapping responsibilities. For example, it suggests that part of the Medical Research Council's (MRC's) Radiobiology Laboratory and the National Radiological Protection Board should be brought together.

Although the team has declined to put any figures on either the costs of such mergers or on the number of jobs that might be lost — and Levene claims that rationalization does not necessarily mean cuts — he admits that "there might be cuts; it depends on what happens during the final decision-making process".

Similarly, some of the individual proposals for mergers are likely to meet with resistance. One is that responsibility for the Roslin Institute in Midlothian, an important centre for animal genetics, be transferred from the BBSRC to the Scottish Office.

In general, however, the scrutiny team's proposals have been given a cautious welcome by many of those who will be most directly affected, and had previously been concerned that the government might be tempted to take more radical measures.

Tom Blundell, for example, chief executive of the BBSRC, says that the report represents an "endorsement" of the efforts the research council has itself been making to rationalize its activities in recent years.

Blundell also claims that the team's report acknowledges that the need for a strategic overview on research means that the 'customer-contractor' relationship introduced by Lord Rothschild in the early 1970s cannot be applied "in an undiluted form" to the relationship between research councils and their institutes.

The strongest reaction has come from the Institute of Professional, Managers and Specialists, the trade union which represents 31,000 scientists working in government laboratories covered by the report.

Valerie Ellis, the union's assistant general secretary, warns that privatization remains enshrined "as a long-term aim for all establishments". But she also acknowledges that scientists will be relieved that their worst fears of a mass privatization programme have not been realized.

William Waldegrave, the minister for science, has said that the government will only decide on what action to take after a four-month consultation period, due to end in November. And in case neither of its two main proposals are acceptable, the scrutiny team has also recommended a third 'fallback' solution — the appointment of two 'directors of rationalization' charged with carrying out the government's aims through less confrontational (and no doubt less expensive) techniques. **David Dickson**



Levene: cutbacks not ruled out.

German research reactor gets local backing

Munich. Plans for the Technical University of Munich's controversial research reactor FRM-II, which will use highly enriched uranium to produce a high-energy neutron source for researchers, have been endorsed by the state government of Bavaria.

Last week, the state agreed to pay around DM300 million (US\$428 million) of the project's estimated cost of DM680 million, and approved Siemens as the main contractor. It also agreed to spend DM1.5 million on preparing the site of the reactor, due to be built at Garching near Munich; part of this cost will be for the construction of a fence to keep out objectors.

The consensus agreement, pushed through in the last session of the government's finance committee before the summer recess, has been heavily criticized by Germany's Social Democrat and Green parties, partly because of its uncertain finances and partly because of uncertainties over the source of the highly enriched uranium.

Financially, the agreement depends on the federal government agreeing to increase its promised contribution to the project, which was based on sharing equally the costs when they were estimated to be only DM525 million earlier this year.

This outcome is by no means guaranteed. Nor has it been agreed who will pay for any future cost rises in the project, which could rise to more than DM1 billion by the time the project is completed.

A more general concern is that FRM-II may end up as a high-cost white elephant, unable to obtain the fuel that it will need. In building the reactor, Germany would break ranks with other nations that are complying with a US initiative to convert research reactors to the use of low enriched uranium, whose threat of contributing to proliferation of nuclear weapons is perceived as being lower (see *Nature* 369, 89; 1994).

The United States controls the world's supply of highly enriched uranium for research reactors, and now supplies it only to reactors that cannot physically be converted to low enriched uranium. The Germans are unwilling to change the design of FRM-II because of the extra running costs involved.

The United States now seems unlikely to shift its position. In May, it sent a statement to the German government confirming that "the US would not supply any new foreign research reactor, such as the planned Garching reactor to be built at the University of Munich".

Klaus Böning, the physicist who heads the FRM-II project, says that the US stand is unfair as Germany, which is a signatory to the Nuclear Non-Proliferation Treaty, would subject all its nuclear fuel to international scrutiny, and would therefore pose no threat.

Now, he says, Germany is depending on the European nuclear supply agency Euratom

to find a solution. Euratom is said to be looking at the United Kingdom, France and Russia as possible local suppliers of highly enriched uranium. Euratom is known already to have visited Moscow, accompanied by a representative from the FRM-II project in Garching as observer.

The outcome of these meetings is not known. But the United States has confirmed that it is also negotiating with the three countries involved to persuade them not to re-export highly enriched uranium originating in the United States.

Although the FRM-II has still to receive a nuclear licence, this is expected to be granted next year despite vociferous opposition. Given the political and financial problems that the reactor has generated, Bavaria's motives for pushing the project so hard are unclear, although one theory is that the building of the reactor will provide a major boost to local high-technology industries.

The FRM-II, designed by the Munich-based company Siemens, has a unique design described by Paul Leventhal, president of the US-based Nuclear Control Institute, at a public meeting in Munich last month, as "the sports car version of the research reactor". He added: "It is small, powerful and a viable export item"; that was why it was a "particularly dangerous" threat to US efforts to control the spread of enriched fuel.

Scientists have been continuing to take sides in the dispute. A protest letter signed by 50 German physicists and published at the end of May was followed two weeks later by a letter of support signed by more than 300 scientists.

While openly bullish on the outcome, Bavaria has been keeping its options open. In agreeing the project last week, the state government reserved its right to pull out of the agreement "if financial or technical changes are too serious". **Alison Abbott**

Neutron sources need collaboration

Paris. Better medium- and long-term planning of neutron sources is needed, according to a report submitted last week to the 'megascience' forum of the Organization for Economic Cooperation and Development (OECD) on synchrotron radiation sources and neutron beams.

In particular, many neutron sources in Europe are "too small, underfunded and underused", according to Peter Tindemans the chairman of the forum. The task, he says, is to find agreement on which sources should be closed, and on what timescale, and to arrange multilateral funding to concentrate resources on those remaining.

Pressure is growing for greater international cooperation in neutron beams and synchrotron radiation sources because of the growing costs of the machines — up to US\$2 billion — and the need for facilities to meet the varied requirements on small users from different disciplines.

Last week's meeting agreed that one model of cooperation is that developed among the three big third-generation synchrotrons, namely the European Synchrotron Radiation Facility (ESRF), the US Advanced Photon Source (APS) and the Japanese Spring-8.

This model could be applied to smaller neutron and synchrotron facilities, says the report. It also suggests that the United States and Europe should take the opportunity to coordinate from the outset the construction of the proposed US advanced neutron source (ANS) and the proposed European spallation source (ESS).

According to Tindemans, the United States has agreed to hold a joint conference

with the OECD in November on next-generation spallation sources costing between US\$0.5 billion and US\$1 billion.

The forum also discussed the conclusions of an expert report on particle physics. The two main items on the particle physicists' shopping list are a high-energy hadron collider — of which the large hadron collider (LHC) is now the only one currently being planned — and subsequently a large electron-positron linear collider (known as the next linear collider, or NLC).

Tindemans says that the megascience forum is trying to persuade governments to make NLC an international project (participating institutions have produced a memorandum of understanding on joint research and development). He is concerned that otherwise the NLC may suffer the same fate as the Superconducting Super Collider (SSC), recently killed off in the US Congress (see *Nature* 365, 773; 1993).

He stresses the importance of scientists and politicians talking together and suggests, for example, that the membership of the International Committee for Future Accelerators should be enlarged, with government officials invited to participate more frequently than once every three years.

The megascience forum also discussed how best to ensure its own future, which is due to be decided at a ministerial meeting next year. The forum itself would like to be given responsibility for monitoring and catalysing international scientific collaboration, and more political clout through the increased participation of high-level government officials in its meetings.

Declan Butler

Authorship and misconduct

SIR — The leading article "What to do about scientific misconduct"¹ arrived in Canada at the same time as headlines in a major national newspaper announced the results of an inquiry into a professor's shooting rampage at Montreal's Concordia University². The inquiry concluded that there was some truth to his claim that his senior colleagues had engaged in "misappropriation of authorial credit", and pointed to the "risk that a production-driven research culture will tempt people to engage not only in undesirable modalities of research, but also in actual falsification and fraud". This view and your remedy, that there should be a "weakening of links between personal success and publication", do not really get to the heart of the problem, and, if misconstrued, might even make the problem worse.

There is nothing wrong with a link between personal success and publication. The problem is that quality evaluation in research is highly error-prone. Any system that is error-prone has to be designed taking error-proneness into account. Conventional peer-review does not do this. An alternative, bicameral review, has been proposed³⁻⁵. The golden rules for working in error-prone environments are (1) use the most objective parameters, and (2) hedge your bets. Under bicameral review, research grant applications are divided into two parts, prospective and retrospective. The prospective part (work proposed) is short and is evaluated by the granting agency, solely on the basis of budget justification. The retrospective part (track record) is evaluated by peers who rate it on the basis of productivity per research dollar received. Finally, rather than a sharp cut-off point below which no funds are awarded, a sliding scale is used.

One cannot get rid of competition. But, if implemented, bicameral review might, over the years, decrease the competitive pressure on researchers and enhance communication and collaboration. Civility would return to the research enterprise.

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SIR — As the importance given to publications is central to fraud and other forms of scientific misconduct, the suggestion¹ that the link between papers and personal success should be weakened is a logical step. Indeed, career advancement for scientists depends heavily on the number of publications regardless of the first author/co-author ratio. Restoring the original meaning to the word 'author' would encourage scientists to concentrate on their

own work rather than to search for convenient collaborations.

Tenure committees and granting agencies should concentrate on first or single author papers, and reviewers of manuscripts and research projects should promote responsible authorship. An individual's name becomes known by appearing in that first position and not among the so-called co-authors. This suggestion conforms to previous proposals for fixing an annual limit to papers per scientist or evaluating the career of an individual through a few articles. The cynical and seemingly robust objection that junior scientists would never appear as first authors can be overcome provided they receive appropriate advice and protection from scientific institutions and research councils.

Despite a possible increase in the disputes about who should go first, a proper use of the word author would lead to a more realistic judgement of personal creativity, an attenuation of the prevalent curricular inflation and article devaluation and an acknowledgement of the secondary role of co-authors. This in turn will result in shorter authors' lists with a positive impact on the scientific literature (less space used, more ease). That co-authorship is indeed secondary is illustrated by its widespread use as a token to pay for any contribution/favour or to flatter a superior.

In line with the earlier pungent comments by DeFelice⁶, it can be said that the scientific establishment has resisted criticism of its behaviour patterns and publication trends because most scientists automatically close ranks to protect themselves. It is clear, however, that the publication game rules need to be reappraised in order to rescue the honour of the scientific profession — entrepreneurial science notwithstanding.

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SIR — You ask how we can weaken the link between a researcher's success and his or her list of publications⁷. One way to do so would be to reopen the debate about the connection between spurious co-authorship and scientific fraud⁶. The pressure to produce a tidy result is enormous. Thus, when a laboratory chief requires data for grants or for lectures, the temptation to edit naturally arises. The best results for these purposes are those that fit in with other data or those that anticipate a major advance. The last thing a subordinate wants to find or a laboratory

chief wants to hear is something offbeat or discouraging. If laboratory chiefs become co-authors of data they are not familiar with, which is too often the case, fraud can result. Data editing, indirectly encouraged, goes undetected. Should the laboratory chief invite a colleague on the fringe of the work to become an author, the potential for fraud increases. This common practice introduces another voice for what the data should be rather than what they are, another vote for consensus (or discovery) even if there is none.

If you doubt that this happens, invite comments from graduate students, post-docs and senior associates from big laboratories. Spurious co-authorship is a major cause of scientific misconduct, and even its innocent or well-meaning forms are harmful to the community.

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1. *Nature* **369**, 261–262 (1994).
2. Mackie, R. *Globe and Mail* 8 June (1994).
3. Forsdyke, D. R. *Lancet* **2**, 1382–1384 (1989).
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5. Forsdyke, D. R. *Accountability in Research* **2**, 237–241 (1993).
6. DeFelice, L. J. *Nature* **353**, 104 (1991).
7. Maddox, J. *Nature* **369**, 353 (1994).

Not so humane

SIR — We would like to bring to your attention a patent violation of animal rights, paradoxically caused by extreme human care.

The episode took place last January in an Italian medical institute, where a precious primate was kept in temporary custody. The specimen was an adult female, in the latest stage of pregnancy. Because of the considerable value of both mother and fetus, labour was monitored throughout by the use of a cardiocograph. For this purpose, the mother was restrained in an uncomfortable supine position. For greater safety, uterine dilation was monitored by manual inspection, about 6 times an hour, each time causing evident pain to the animal. Finally, just before delivery, episiotomy was performed to reduce the risks of delivery.

Both mother and baby are now perfectly well, but we believe that this and similar cases should be brought to the attention of whoever cares for animal welfare.

We certify that what is reported is true: *one of us is the primate in question.*

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Population density and wealth

SIR — Since the first edition of Malthus's *Essay on Population*, conventional wisdom has stated that more people mean less goods for each person, and that, as population grows, poverty inevitably increases. Although this may certainly be the case for bacteria or donkeys, for human beings the situation may be quite different. Since the time of Malthus, the world's human population has increased 6.6-fold, but ordinary people certainly seem more wealthy now than in the early nineteenth century. It may be argued, however, that this may be due only to the great increase in wealth in the industrially developed countries which, through averaging, blurs the situation in most other countries, where the negative relationship of population to wealth may be valid. To have a sharper picture of this problem, a country-to-country correlation between these factors is needed.

Until recently, this has been difficult to achieve, as prices of the same goods and services vary greatly, and currency exchange rates are frequently distorted by 'official' rates that may lag behind market rates by more than one order of magnitude. Recently, the United Nations has introduced the International Comparisons Program (ICP), which makes useful inter-country comparisons of per caput gross domestic product (GDP).

We have compared per caput GDPs with population density for the 108 countries for which ICP data are available¹. These countries represent 90.4 per cent of the world's population¹. When comparison is done on a per country basis (figure, upper panel), there is a small positive

correlation ($r = 0.192$) that has a $P < 0.05$. When the correlation is done weighing the points according to each country's total population (lower panel), the correlation is very slightly negative ($r = -0.048$), and not significantly different from zero ($P < 0.7$).

This study, therefore, does not support the commonly held belief that more densely populated countries are poorer than those that are more sparsely inhabited.

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1. *World Development Report 1992*, Table 30 (World Bank/Oxford University Press, 1992).
2. *World Resources 1990–1991*, Table 17.1 (World Resources Institute, Spanish Edition, Instituto Panamericano de Geografía e Historia, México, 1991)

Science and anti-science

SIR — The leading article "Defending science against anti-science" (*Nature* 368, 185; 1994) contained misleading statements that flowed into over-extended and potentially offensive conclusions. The arguments fall apart where they hinge on the scientific method. In other words, if experimentation or controlled observations are not the bases of the conclusions reached in the leading article, then the subject of the attack has as much validity as do the baseless arguments.

It is possible that "astrology is a pack of lies". However, it has never been established that this is the case using the tools of astronomy, statistics and psychology. If applications of our scientific techniques have not invalidated astrology, then dismissing it is similar to the Catholic Church's historic treatment of Galileo. According to the article, "truth is not absolute, but rather a series of working hypotheses. . . ." If this is so, then how can it be a "plain fact" that non-mainstream practices, such as astrology, "cannot be correct"?

My understanding of the content of much of the article was that 'non-science' is actually the subject of this vituperative attack. Although the "forces of anti-science" no doubt exist, "other mumbo-jumbo such as faith-healing, water divin-

ing and spiritualism" hardly qualify as actively threatening the practice of modern science. There have been many distasteful attempts in European history to denounce non-mainstream ways of thinking, as well as campaigns to eradicate the practitioners of such unthinkably objectionable arts as astrology.

As a scientist, I find it quite chilling that you would describe any scientist-critic as an "insidious" threat to the theory and practice of modern science. The greatest advances in science have come about through criticism and proposals that are radical, not conforming to the accepted standards of the day. To reject alternative explanations at any point in our cultural evolution is tantamount to saying: 'at this point we know absolutely the true nature of the universe'. This is unlikely to ever be true. Please stop encouraging limitations on "the enhancement of the general enlightenment".

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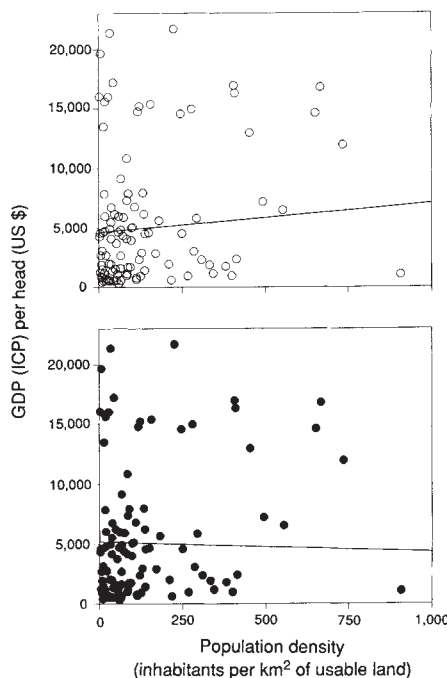
SIR — John Maddox is concerned about the forces of anti-science, and he asks for a polemicist to take up the fight against them. His outrage, although I do not share it, deserves a response.

Certain philosophies are inherently paradoxical. Advocates of democracy, for instance, are surely frustrated that they must occasionally tolerate speeches for tyranny and bigotry. Such frustration is a side-effect of democracy, and may be an essential aspect of democracy. The paradox is that stifling undemocratic speeches puts one in the undemocratic camp.

Advocates of science must also invite criticism, as criticism of the basic tenets of science is the only way in which science can progress. Must this criticism come only from the true believers? That would produce an ultimately sterile philosophy. Science, in the mirror of anti-science, has its blemishes magnified, and that is good for science. Maddox asks for someone to take up the cudgel against anti-science, but that cudgel would strike against science itself, in breaking the mirror. I believe that the restraint that Maddox criticizes in Holton's anti-anti-science stance is an essential aspect of science.

If anyone is unconvinced that Maddox's argument stands more on the side of anti-science than science, one has only to ask what, if anything, the approaching end of the millennium has to do with the need for science to describe itself. That sounds like an astrological casting to me.

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Market economics in Russian research

Russian research institutes are being compelled to live by their wits, assembling annual budgets from a variety of sources and attracting young people only if they can pay them enough.

Moscow. Everything has changed and everything is the same. That is the first impression of Moscow after the long interval of a year. And the evidence of permanence is the more conspicuous. The Kremlin is still there, of course. So is the sprinkling of gold-domed churches, while the White House, the parliament building attacked by government tanks just nine months ago, has been patched up at unwonted speed. The people in the streets are less drably dressed than a year ago. Signs of the mafia supposed to have a stranglehold on emergent Russian business are not much in evidence. That is the good news.

The signs of change are mostly superficial. Hotels and many shops now denominate the prices of what they have to sell in US dollars, but then prominently display a notice proclaiming "RATE = 2100" (where the number changes from one day to the next) so that customers can calculate what they must pay in rubles, theoretically the only legal tender now. This is a neat accommodation of inflation, now between 5 and 7 per cent a month. (The dollar rate reached 2,000 last week, six months ahead of January's prediction for the end of the year.)

But the urban road network is disintegrating faster than it can be repaired. In several centre-city streets, potholes must account for half the surface area. On some streets, the surface has been removed in preparation for repair, with the result that heavy iron manhole covers project up to 10 cm above the driving surface, making simple journeys resemble a giant chicane. Some blame the damage on last October's visitation of tanks, others the sheer weight of traffic. Either is a sufficient explanation.

Meanwhile, beneath the surface, Russia's traditionally rich literary life is fast being eroded. Throughout the Soviet period, the country was amply endowed with monthly literary magazines, *Novy Mir* for example, which were a good read even in Stalin's time and which, with *glasnost* in 1986, published previously forbidden texts. Between then and the end of the decade, these magazines were eagerly and widely read and their contents endlessly discussed. But no longer. With the arrival of 'the market', they have become unaffordably expensive. That is one problem. The other is that people no longer have the time to read them. The old habit of curling up in the corner of the laboratory with that month's copy of *Novy Mir* is no longer acceptable.

Science is in much the same case, but for

different reasons. At half a dozen institutes last week, the chief anxiety was the recruitment of able young people. The old routine of assigning able students to institutes in the last one or two years of their first degree course, in the expectation that a well performed candidate's (PhD) course would be followed by permanent employment, has too often been frustrated by young people's preference for better pay.

Pay now depends on an institute's success in recruiting funds from outside the usual channels, the Russian Academy of Sciences in particular. At most research institutes, the academy's share of the budget is down to 30 per cent. Other sources of funds are the science ministry, other government ministries (the Ministry of Atomic Energy, for example), the Soros Funds and the Russian National Research Foundation. Institutes able to make scientific instruments for collaborators abroad do so with a will.

The principle that research institutes must play the market to remain healthy seems well established. The importance, and thus the influence, of the academy is correspondingly reduced. But many institute directors find the need to scabble around for funds distasteful, even demeaning. Theirs are the institutes that languish.

In the circumstances, it is remarkable that so much enthusiasm abounds. Take, for example, the complex of four research institutes at Troitsk, some 40 km south of Moscow. (If Novosibirsk in Siberia is a 'science city' and Pushchino, further south in Moscow Region, is a 'science town', Troitsk is a 'science village' and may soon be a 'science suburb'.) The Institute of Terrestrial Magnetism and the Ionosphere, or IZMIRAN (one of the four at Troitsk), began life in 1940 as an ionospheric laboratory, which in the 1960s became a general solar-terrestrial laboratory.

In the early 1970s, it made an international reputation by injecting beams of charged particles into the Earth's magnetic field from rockets and spacecraft, creating artificial aurorae incidentally to the study of the motion of charged particles in the Earth's magnetic field. Now it has at least one instrument on most Soviet and many international interplanetary spacecraft. Its latest boast is a satellite (KORONAS C) designed to measure X-ray emission from the Sun, from 30 nm (just beyond the ultraviolet) to the γ -ray region. It has been the prime mover in the design of an international project in which the ionospheric effects of electron

and Xe-ion beams from one satellite are measured at another, whose distance from the source can be varied from a few metres to a few kilometres.

By Russian standards, all this is accomplished economically, by no more than 350 established scientists. Apart from the spacecraft work, there are data to collect from all over Russia, a nonmagnetic ship (a sailing vessel) and a nonmagnetic aircraft to keep in service and instruments to manufacture for all and sundry. The job is done with considerable *élan*; the snag is that there are too few research students, perhaps because the mean salary is only 100,000 rubles a month, and the average age in the laboratory in what is really a young person's game is 54.

In the Russian tradition, the laboratory also holds some daring views not shared elsewhere. It believes, for example, that there are regional disturbances of the Earth's geomagnetic field up to two days ahead of major earthquakes and that people are sensitive to magnetic storms, which are said to predispose them to heart attacks. It has already installed magnetometers at three Moscow clinics in the hope that prophylactic strategies can be worked out. The director of IZMIRAN, Professor V. N. Oraevsky, claims epidemiological evidence based on the coincidence of cardiac trouble with the onset of magnetic storms. He promised last week to write with further details.

None of that detracts from the quality of the institute's work, but rather is a measure of how Russian science still suffers from the traditions of self-sufficiency. (Isolation is still fearfully reinforced by the continuing and despairing shortage of journals from elsewhere.) Daring ideas can hang in the air for years on end, untested by the international community. There is also ample evidence that much excellent work is effectively denied international recognition by publication in Russian journals which, even when in English, circulate poorly and slowly elsewhere in the world and, when in Russian, are translated at a snail's pace.

Despite the growing influence of 'the market' on research affairs, it is plain that Russian science is not yet fully alive to the need to market its achievements by the now-standard routes of rigorous peer review followed by modest advocacy of their importance on the international scene, at meetings for example. The belief persists that all publications are equal, equipotent pebbles on the beach of discovery. When last was that the case?

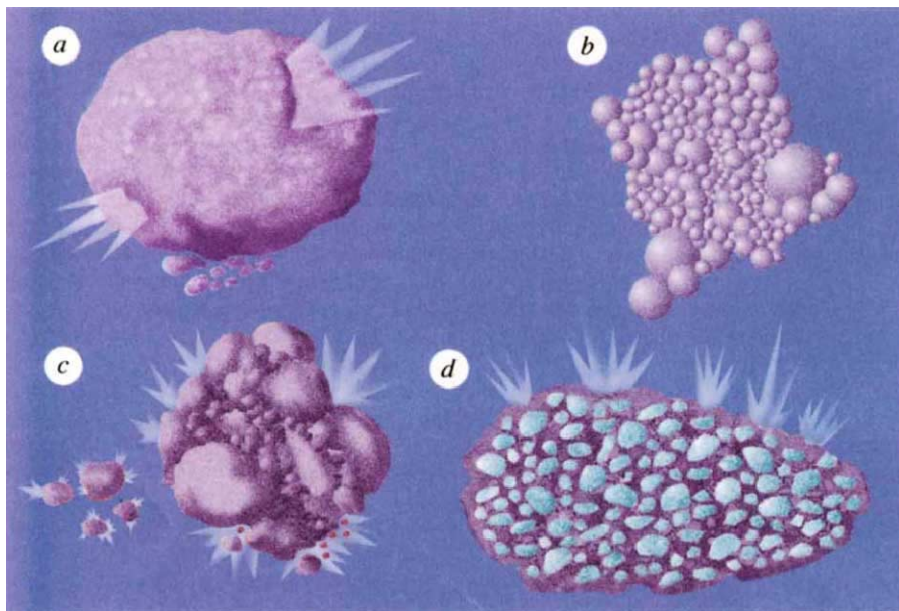
John Maddox

The Big Fizzle is coming

Paul Weissman

How will the fragments of comet Shoemaker-Levy 9 meet their end — with a bang or a whimper? That is the question on everyone's mind as the icy fragments rush towards their cosmic rendezvous with Jupiter, beginning on 16 July. Will Jupiter's atmosphere be torn with massive explosions, each greater than the sum of all the nuclear weapons on Earth, or will it

tions show the nucleus of tightly packed snowballs being torn apart by Jupiter's gravity during the close approach, the hundreds or thousands of snowballs stretching into a long column in space. But as the column lengthens and moves away from Jupiter, the individual snowballs begin to clump together because of their own self-gravity. The truly amazing result



Four suggested structures for a comet nucleus, adapted from ref. 10. *a*, The nucleus as an icy conglomerate, *b* a fractal structure, *c* a 'primordial rubble pile', as Asphaug and Benz¹ assume for comet Shoemaker-Levy 9; and *d* the 'icy-glue' model.

be a giant fizzle? We are about to find out.

Whatever the outcome, the breakup of comet Shoemaker-Levy 9 has provided fresh clues as to the structure of cometary nuclei and their bulk density. One fascinating example is to be found in the paper by Eric Asphaug and Willy Benz on page 120 of this issue¹. Asphaug and Benz used a high-speed computer workstation to model the breakup of Shoemaker-Levy 9 when it passed within Jupiter's Roche limit two years ago. They assumed that the comet was a 'primordial rubble pile', a collection of hundreds to thousands of dirty snowballs, held together only by their own self-gravity.

This model for comets was independently proposed nearly a decade ago by myself², and by Bertram Donn and David Hughes³, who referred to their idea as the 'fractal model'. An improved description of how such 50-metre-diameter dirty snowballs (or more aptly, frozen mudballs) might form in the primordial solar nebula and then come together to form kilometre-sized nuclei was recently provided by Stuart Weidenschilling⁴.

Asphaug and Benz's dynamical simula-

ions show the nucleus of tightly packed snowballs being torn apart by Jupiter's gravity during the close approach, the hundreds or thousands of snowballs stretching into a long column in space. But as the column lengthens and moves away from Jupiter, the individual snowballs begin to clump together because of their own self-gravity. The truly amazing result

is that the number of clumps formed appears to be a function of the density of the individual snowballs. At a density less than 0.4 g cm^{-3} , no clumping occurs; at a density of 2.4 g cm^{-3} all the snowballs come back together to form a single body. But at intermediate values, in particular between 0.4 and 0.9 g cm^{-3} , the snowballs form 15 to 20 clumps. Comet Shoemaker-Levy 9 consisted of 21 individual nuclei when it was discovered last year. (Note: the densities quoted here are those of the individual snowballs; Asphaug and Benz use the bulk density of Shoemaker-Levy 9 before it broke up, which is about 27 per cent less because of the voids between the packed snowballs.)

Results are modified if the original comet nucleus was rotating. Asphaug and Benz's simulations rule out a retrograde rotation, because the snowballs then form a large central clump and smaller outlying clumps; this was not observed for Shoemaker-Levy 9. But if the comet had a prograde rotation, 15–20 clumps are obtained if the density of the snowballs is higher, perhaps 1.3 g cm^{-3} . Asphaug and Benz's results also suggest that the origin-

al comet nucleus was fairly small, at most 1.5 km in diameter, in agreement with work by Scotti and Melosh⁵.

Past estimates of the bulk density of cometary nuclei have ranged from 0.1 to 1.3 g cm^{-3} , based on comparisons of the predicted effects of gases jetting from the sunlit surface of comet Halley with detailed observations of Halley's orbital motion^{6–8}. But the many free parameters in such comparisons make the estimates highly uncertain. More recently, meteoriticists have measured the density of microscopic cometary dust grains recovered by U-2 aircraft high in the Earth's atmosphere⁹; these values are typically between 1 and 2 g cm^{-3} . Asphaug and Benz's results clearly rule out the lower range of values from the estimates of jetting forces, but may be in conflict with some of the higher values from the cometary dust grains.

A question not answered by Asphaug and Benz is whether the individual dirty snowballs in each clump of Shoemaker-Levy 9 have reaccreted into a single body, or whether they are only gravitationally bound dynamical swarms, like bees buzzing around a hive. Several of the clumps have been observed to split, well away from Jupiter's tidal pull, suggesting that within each clump several sub-nuclei may reaccrete, but that a single solid body did not form. Other clumps have dissipated completely with time, suggesting that the snowball swarms don't reaccrete and instead dynamically disperse or sublimate away.

What does this say about the coming impacts on Jupiter? As the clumps approach for their final plunge into the atmosphere at 60 km s^{-1} , Jupiter's gravity will again pull them apart. Rather than hitting as a single solid body, they will probably come in as an elongated shotgun blast of smaller pellets. Because of Jupiter's rapid rotation, the impact sites will be spread in longitude, like machine gun bullets lacing into a moving target. Each snowball will individually ablate and burn up like a meteor in Jupiter's upper atmosphere. Lacking the momentum and the structural integrity of a single solid body, they will probably not penetrate deeper into the atmosphere, where they might explode with many thousands of megatons of energy.

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Thus the giant impacts will produce a spectacular meteor shower of bright bolides, but not the massive fireball explosions that have been predicted by some researchers. The impacts will be a cosmic fizzle. The cometary meteors may resemble the bolide which exploded harmlessly at 25–34 km altitude over the south Pacific on 1 February this year, with an estimated yield of 15–20 kilotons. The Shoemaker–Levy 9 snowball explosions may be closer to about 30 megatons each, but still far less than the 100,000 megaton explosions that some have predicted.

Nevertheless, Shoemaker–Levy 9's legacy will probably be an improved understanding of the nature of cometary nuclei. It will provide a dramatic confirmation of the primordial rubble pile and fractal models, and will provide the first definitive bounds on the bulk density of cometary nuclei. Then again, maybe it won't. □

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EPIDEMIOLOGY

A theory of malaria vaccination

Christopher Dye and Geoffrey Targett

COULD malaria vaccines eradicate *Plasmodium falciparum* from its stronghold in tropical Africa? While trials of Manuel Patarroyo's Spf66 vaccine¹ are under way in Tanzania^{2,3}, The Gambia and Thailand, following partial successes in South America⁴, Gupta *et al.*⁵ have published a stimulating theoretical analysis which suggests that the eradication of malaria by vaccination might be nothing like as difficult as imagined. They also propose that their theory can explain the distribution of different clinical forms of malaria with age⁶, and the diversity and dynamics of different parasite strains⁷.

The argument about vaccination builds on the logic of the campaign against measles, mumps and rubella. Simple epidemiological theory says that the critical fraction (p) of people to be immunized with a combined vaccine (MMR) to ensure eradication of all three pathogens is determined by the infection that spreads most quickly through a population; that is, by the one with the largest basic case reproduction number, R_0 . In the case of MMR, this is measles, with R_0 of around 15, which implies that $p > 1 - 1/R_0 \approx 0.93$. Gupta *et al.*⁵ point out that, if a population of malaria parasites consists of a collection of pathogens or strains that have the same properties as common childhood viruses, then vaccine coverage would be determined by the strain with largest R_0 , rather than by the R_0 of the whole parasite population. While estimates of the latter have been as high as 100, the former could be much lower.

Analogy

The MMR analogy is a useful one in the context of this theory because it makes clear the defining characteristics of a 'strain' of *P. falciparum*. Strains are not merely antigenic types; rather they must fulfil two particular conditions. First, infection with each strain should generate fully protective, lifelong immunity to that

strain. Immunity here means that an individual should be protected against becoming infectious following further exposures to the parasite; protection of this kind may or may not be associated with clinical immunity. Second, there must be no cross-protection between strains.

Are there strains of *P. falciparum* that satisfy these two conditions? Gupta *et al.*⁵ have identified the neoantigens (PIESAs) produced on the surface of infected erythrocytes as markers of specific strains. As they acknowledge, the observation that these antigens generate a long-lasting antibody response does not imply durable protective immunity. The only evidence so far that PIESAs stimulate a protective response comes from work by Marsh *et al.*⁸ in The Gambia. They found that clinical episodes were fewer, although protection was neither complete nor proven to be durable. They also did not address directly the more relevant but difficult problem of protection against becoming infectious (producing viable gametocytes which are transmissible to mosquitoes), although there was no evidence in cross-sectional data that antibodies to PIESAs prevented parasitaemia.

Although a degree of clinical protection may be associated with strain- or variant-specific antibody responses, these antibody responses may in turn be associated with common or cross-reacting T-cell epitopes that progressively enhance the speed of response to new variants⁹. Gupta and colleagues⁵ do actually acknowledge that cross-protection may have a part to play, and they use the idea in their other recent papers to explain why children with cerebral malaria tend to be older than children suffering from severe malarial anaemia⁶, and to show how oscillations in malaria incidence could be driven by immunity rather than by fluctuations in external factors, such as mosquito density⁷. Whether or not these explana-

tions are correct is debatable; their relevance here is that they suggest a bigger vaccination problem because cross-protection implies higher effective R_0 values.

Test

One test for cross-protection calculates the expected number of people in different age classes who have been exposed to different strains, assuming independent transmission and some distribution of reproduction numbers across strains. Gupta *et al.*⁵ found that expected and observed numbers matched quite closely when five strains studied in Papua New Guinea were each given the same R_0 of about 7. This is a useful numerical demonstration of the way in which R_0 can appear quite small, but it must be seen as no more than illustrative for two reasons. First, if exposure to each strain does not lead to effective protection, then the question of cross-immunity is irrelevant. Second, the supposition that each strain has roughly the same low R_0 is not yet backed by detailed calculations of the incidence rates of a large collection of strains. Parity of R_0 values is not in any case essential for their argument; the strains could have R_0 values which vary from low to very low. Indeed, this is one proposition made^{6,10} to explain how insecticide-impregnated bednets can reduce malarial disease without reducing the prevalence of infection.

Many malariologists with control experience will doubt that R_0 in highly endemic areas is as low as Gupta *et al.* suggest. Notwithstanding the effort being devoted to vaccine development, attacking the mosquito population continues to be a mainstay of malaria control. The criterion for eradication by vector control is essentially the same as that for vaccination; p can also be interpreted as the critical fraction by which the mosquito population must be reduced. If $R_0 = 7$, say, then $p > 0.86$. Magesa *et al.*¹¹ satisfied this condition in a DDT trial in Tanzania, but their success was accompanied by no more than a small reduction in *P. falciparum* incidence, and no detectable reduction in prevalence over the following 12 months. The implication is that R_0 was effectively much higher than 7 in this Tanzanian village.

Even if a *P. falciparum* population does

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consist of a collection of strains, all with low R_0 , the MMR strategy requires a vaccine which will protect against all of them (vector control, by contrast, necessarily attacks all strains). Gupta *et al.*⁵ propose vaccinating against conserved epitopes which, to satisfy the condition that there must be no cross-protection between strains, must be of low immunogenicity on natural exposure. This does seem to be a way forward: success may come from artificially raising the response to common epitopes, to which natural immune responses are rare or suppressed.

The first of Gupta's three recent papers⁵ describes an elegant and optimistic theory of malaria control, but its relevance to *P. falciparum* is far from proven.

PROTEIN FOLDING

Chaperoning nascent proteins

R. John Ellis

POLYPEPTIDE chains are synthesized inside cells by the sequential joining of amino acids to form linear molecules that fold into precise shapes to produce functional proteins. Experiments reported by Frydman *et al.* on page 111 of this issue¹ shed new light on the relation between chain elongation and chain folding. They suggest that several different molecular chaperones act together to control folding while the nascent chains are still elongating.

Protein folding is arguably the single most important process in biology as it produces the three-dimensional structures responsible for the specific properties of proteins. These structures are destroyed by denaturing agents that disrupt non-covalent bonds, but Anfinsen demonstrated more than twenty years ago that some pure denatured proteins will refold into the correct shapes if the concentration of denaturant is subsequently reduced². Refolding is spontaneous and requires no other macromolecules or energy expenditure. Although the rules that ensure that a given aminoacyl sequence folds into a particular shape are unknown, the refolding experiments imply that there is no need to invoke any more complex process for protein folding inside cells. This principle of protein self-assembly is a logical corollary of the Central Dogma of molecular biology, as the only genetic information for proteins resides in the linear sequences of nucleotides that determine the linear sequences of the amino acids in the polypeptide chains.

As so often happens in biology, this logical simplicity is being complicated by subsequent discoveries. More recent evidence suggests that protein folding

Besides, although eradication and the maintenance of herd immunity may be long-term goals of vaccination campaigns, these issues are not the prime concern of the current trials of Patarroyo's vaccine. The priority is to establish whether the vaccine can effectively immunize individuals (rather than populations) in areas where people are exposed to as many as 300 infective mosquito bites per year. We shall begin to know the answer when the double-blind code of the Tanzanian trial is cracked this August. □

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in the cell is neither spontaneous nor energy-independent, but is controlled by pre-existing proteins acting as molecular chaperones³. The discovery of the ubiquitous subgroup of molecular chaperones called the chaperonins⁴ has sparked a continuing wave of repetitions of the Anfinsen refolding experiment with pure proteins, but modified by the addition of molecular chaperones to the refolding buffer.

Such 'neoAnfinsen' experiments suggest that the chaperonins improve the yield of correctly folded proteins, not by violating the principle of protein self-assembly, but by suppressing and reversing chain interactions that produce incorrectly folded proteins⁵. These incorrect interactions are more prevalent in the complex and highly concentrated intracellular environment than in a dilute solution of pure refolding protein, and the chaperonins presumably evolved to combat them; plausible models for how they do this are being discussed^{5,6}. But these experiments are still remote from the conditions that occur inside cells, so their conclusions need to be checked in systems closer to the *in vivo* situation. Do chaperones bind to chains before or after elongation is complete, and when does folding occur? Direct studies of protein folding in intact cells are not yet possible, but crude cell extracts that can synthesize and fold specific proteins on the addition of messenger RNA are available.

In the present study¹, an unfractionated lysate of rabbit reticulocytes (retic lysate) was used to study the relation of elongation to folding. Addition of mRNA for the firefly enzyme luciferase to the retic lysate results in the rapid appearance of full-length luciferase chains of relative

molecular mass 62,000 (62K), but there is a short lag before correctly folded luciferase chains can be detected by measurements of enzymatic activity. A transient 23K fragment, enzymatically inactive but stable to digestion with added proteinase K, is detectable early in the synthetic process, consistent with the folding of an amino-terminal domain of luciferase before synthesis is complete.

Translation of a truncated luciferase mRNA lacking the codons for 11 carboxy-terminal amino acids prevents chain release from the ribosome and no enzymatic activity is detectable. Release of these shortened chains by puromycin causes some enzymatic activity to appear, but the amount is enhanced sevenfold if MgATP is also present. MgATP does not increase the spontaneous refolding of pure denatured luciferase, so its effect in the ribosomal system is interpreted to reflect its well-known ability to reverse the binding of certain molecular chaperones to partially folded polypeptide chains⁵.

Taken together, these results suggest that some folding occurs before synthesis is complete, but that completion of folding requires both release from the ribosome and the activity of molecular chaperones. But are molecular chaperones present in ribosomal-polypeptide chain complexes?

Washed ribosomal pellets from the retic lysate were found by immunoblotting to contain three types of molecular chaperone — Hsp70 (known as DnaK in *Escherichia coli*), Hsp40 (known as DnaJ in *E. coli*), and the TCP-1-containing chaperonin, also called TriC or the CCT complex⁷. All three chaperones are implicated in the folding of polypeptide chains. Hsp70 is known to bind to extended polypeptides in several systems, including nascent chains made by HeLa cells in culture⁸. The TCP-1-containing chaperonin is distantly related to the GroEL chaperonin from *E. coli* and is found in the eukaryotic cytosol⁹. It binds in an ATP-reversible fashion to chains of chicken α - and β -tubulin made in a retic lysate¹⁰, whereas a TCP-1-containing chaperonin purified from retic lysate behaves as a chaperone for refolding pure denatured β -actin chains¹¹.

The new observation¹ is that a high proportion of the incomplete chains of luciferase made during a short incubation of a retic lysate is released from ribosomes in a high molecular weight form (about 1,200K as judged by gel sieving) by the addition of puromycin. This large complex behaves as a uniform entity on non-denaturing polyacrylamide gels, and the nascent chains it contains are immunoprecipitated by antisera to the TCP-1-containing chaperonin. Notably, the same spectrum of incomplete chains is also immunoprecipitated from this complex by antisera to either Hsp40 or Hsp70, while antiserum to Hsp70 coprecipitates TCP-1-

containing chaperonin. This suggests that these three chaperones are present together in association with nascent chains attached to ribosomes, but the structure and composition of this complex are unknown. Are all three chaperones required for efficient folding and is there a defined order of association with nascent chains?

Partial depletion of each chaperone separately from a retic lysate by immunoprecipitation does not inhibit translation but does decrease the recovery of enzymatically active luciferase chains. Addition of purified Hsp70 to an Hsp70-depleted lysate at the start of translation restores the recovery, as does the addition of TCP-1-containing chaperonin to a lysate depleted only of this chaperone. Very short luciferase chains (77 residues) made with truncated mRNA are released by puromycin in the form of a 200K complex containing Hsp70 but not the TCP-1 chaperonin.

These and other results suggest that Hsp70 and Hsp40 bind first to the elongating chain, and function to prevent incorrect interactions before enough chain to form a discrete domain has been made. These small chaperones are necessary but not sufficient for this domain to fold, as folding requires subsequent transfer to the large TCP-1-containing chaperonin before translation is complete. Formation of the completely folded protein occurs only after chain release from the ribosome, however, and also requires the TCP-1-containing chaperonin.

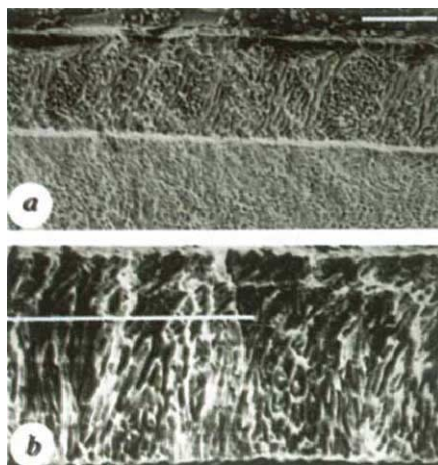
This picture of a highly organized chaperone pathway operating both during and after translation in the eukaryotic cytosol requires confirmation with other proteins and testing by additional methods. The postulated association of the TCP-1-containing chaperonin with nascent chains in intact cells should be demonstrable by pulse-chase immunoprecipitation experiments, and the large complex reported should be visible in the electron microscope. Does the eubacterial GroEL chaperonin also bind to nascent chains? The gap between cell extracts and intact cells is closing, but there is still some distance to go before we understand how protein folding is controlled by chaperones *in vivo*. □

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Roots of rodent radiation

Lawrence J. Flynn

SOME light has been shed on the enigmatic origin of Rodentia in the report on page 134 of this issue of an important new fossil from Inner Mongolia¹. The Rodentia constitute one half of mammalian diversity and mean more biologically than just one of nature's oddities or agricultural and domestic pests. Rodents provide medical subjects, physiological models and molecular resources, and are the subject of many biological experiments, yet the



Scanning electron photomicrographs of longitudinal sections through lower incisors of two Glires from the early Eocene of Mongolia. Both specimens are from the same Tsagan-Khushu quarry from the Bumban Member of the Naran-Bulak beds. *a*, A non-rodent described originally as *Zagmys insolitus*. The top of the photograph is external to the enamel, the lower third is dentine; the enamel shows only one layer and inclined Hunter-Schreger bands. *b*, Rodent enamel, showing two layers; the scale bar is approximately at the division between inner and outer portions. These photographs show the major differences between one- and two-layered enamel, features that are still incompletely surveyed for early Glires. Scale for both represents 0.1 mm; the incisor tip is to the right in each. (Photographs courtesy of H. Burger.)

content and relationships of the group have been continually disputed. This impressive new fossil, which is more than 50 million years old, goes far towards illuminating the roots of the rodent radiation.

The drama of unveiling the origin of this Order of Mammalia is augmented by the provenance of the fossil. Meng and co-workers¹ assembled a small expedition from Beijing to Inner Mongolia. They endured frontier rigour to get to fossiliferous rocks in excess of 50 Myr old. There, in fossil heaven, they recovered a remarkably complete dentition of an entirely new genus of rodents. Even the context of fossilization is extraordinary. The teeth were found inside what the

authors interpret as a coprolite — fossilized excrement. The Palaeogene predator of the teeth's owner is unknown; accumulations of owl pellets 6 Myr old are known from China, but what this early Cenozoic agent of fossilization was is anybody's guess.

The new creature, *Tribosphenomys*, is exciting not only because it is old, but because a rigorous, tandem cladistic analysis employing many taxa and character complexes shows it to be quite primitive. In that it appears to occupy a position basal to other Rodentia, its features can reasonably be evaluated as representing the primitive morphotype for the group. It is this that seems to contradict prevailing wisdom. *Tribosphenomys* has an appropriate tooth formula and jaw shape, but the upper teeth are surprisingly un-rodent-like. They are not squared as in all other rodents; they lack the usual internal hypocone cusp next to the protocone. The few other modern rodents that lack a hypocone (squirrels) still have square teeth because the internal side is expanded. The teeth of *Tribosphenomys* are not internally expanded; given that this rodent is so primitive, it is reasonable to consider, as do Meng *et al.*, that the triangular upper molar could be common to basal Rodentia.

This idea is challenged, however, by the condition of outgroups. Other Glires, including rabbits and an array of early Asiatic forms, and even Macroscelidea (potential lagomorph relatives²) show a hypocone. It seems equally reasonable that hypocones are primitive and *Tribosphenomys* is derived in lacking a hypocone; if so, that could be a linkage with squirrels and Palaeogene paramyids. However, most early Glires show oval molars, with a small hypocone on a shelf below the level of the protocone: transformation from such an intermediate condition either to eliminated or to greatly expanded hypocones would result in two derived states. Careful mapping of this trait in more fossils is required.

Outgroup comparison also tests polarity in a completely different character complex — incisor enamel microstructure. Glires incisors are highly evolved, being over-growing and chisel-like for gnawing. The chisel shape is promoted by the hard enamel being restricted to the anterior side of the relatively softer dentine. The enamel crystallites show a highly organized structure, double-layered in rodents (but see refs 3 and 4), and single-layered in other Glires (see figure).

In a landmark paper, Koenigswald⁵ argued that two layers was primitive, a

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single layer derived. With the realization that the enamel of most early Glires is single-layered⁶, and with a broader sampling of the two-layer character state in basal Rodentia^{3,4}, rodents can probably be considered as derived in their double layering. This has implications for the cladogram of Meng and co-workers. Derived two-layer enamel would be a synapomorphy linking the close relative of rodents *Heomys* with Rodentia, notwithstanding the two layers described for the primitive Glires *Eurymylus*. This strengthens the topology of the cladogram and argues that *Heomys* is indeed related to rodents (if not a member), as originally suggested⁷. In other morphology, the authors find an intriguing connection in optic foramina between *Heomys* and the early rodent *Cocomys*; I would combine this with overall dental similarity to suggest that *Heomys* may be a primitive cocomyid.

Returning to incisor enamel, Meng *et al.* examined an incisor of their new beast. They consider it with some reservation to show a character state (one of three) termed pauciserial. In fact, their figures are insufficient for full description of the enamel, or for the suspicion that Hunter-Schreger bands are absent, which would be unexpected for Glires. These traits also require careful mapping across more taxa. Interestingly, Meng *et al.* adopt the notion that pauciserial is primitive for rodents. Not long ago, this argument was unacceptable because it could not be defended on independent grounds. Recent work⁴ amassing a great deal of comparative data shows that all early rodents are pauciserial, that derived conditions appear later, and that no Neogene rodents are pauciserial. The totality of observations based on large samples is now apparently sufficient in itself to make another polarity extremely unlikely.

Finding more and better fossils will add important new data, and some of the quandaries in interpretation will be sorted out by more comparative work in the laboratory. But it is important not to get too lost in incisive criticisms. This fossil represents a lot of hard work and is indeed a golden nugget. □

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More light on a peculiar galaxy

James M. Moran

THE galaxy IC4553 is relatively undistinguished at optical wavelengths. But it has come under intense scrutiny in the past decade because of its enormous luminosity at far-infrared wavelengths, strong molecular emission and disturbed morphology. Direct measurements on a scale small enough to probe the galaxy's central region are difficult because at the galaxy's distance of 75 megaparsec, an angular resolution of 1 arcsec corresponds to a linear dimension of about 370 parsec. On page 117 of this issue, Lonsdale, Diamond, Smith and Lonsdale¹ describe new observations at radio wavelengths of the OH maser emission made with unprecedented resolution with an array of telescopes operating as a very-long-baseline interferometer (VLBI).

The galaxy, also known as Arp 220 (Arp included it in his catalogue of peculiar galaxies under the category of galaxies with adjacent loops), has a luminosity of about 10^{12} times that of the Sun. When imaged at infrared and radio wavelengths, the system shows two nuclei². There is broad agreement that Arp 220 is a merger of two galaxies, but there are two opinions on the nature of the primary energy source; the suspects are starburst activity and active galactic nuclei that are fuelled by accretion.

The OH molecules in the molecular clouds of the galaxy undergo population inversion in the copious bath of infrared radiation and act as natural maser amplifiers. Lonsdale and his colleagues find that the maser clouds amplify the radiation from very compact parts of the nuclear radio sources by a factor of about a hundred, and they highlight the structure on an unprecedented scale of a few parsecs. Because of the nature of the observations the information is fragmentary, but it gives a glimpse of the exciting prospects of probing the core of this enigmatic object and similar galaxies in detail.

Arp 220 has become the prototype of a new class of galaxy — the ultraluminous infrared galaxy. It emits 98 per cent of its radiation energy at infrared wavelengths, and its spectrum, which peaks at about 100 micrometres, is accurately modelled as arising from dust at a temperature of about 50 K in a volume of diameter 400 parsec (ref. 3). The dust presumably re-radiates energy from an unseen embedded source. The galaxy has two distinct nuclei, both of which are prominent compact synchrotron radio sources. The considerable evidence of massive disturbance includes a chaotic galactic halo, large velocity dispersion and compression of regions of gas and dust. These are unmistakable

signs of a merger of two galactic systems, rather than just tidal interaction with a neighbouring galaxy.

In current ideas of galactic mergers, the stars mingle without significant interaction, whereas molecular clouds collide, producing shock waves, gas compression and bursts of star formation. Proponents of the primacy of star formation point to a high mass and high density of molecular material in the core^{4,5}. On the other hand, the merger may also trigger activity in the nuclei, fuelled by infalling material from the interstellar medium and possibly from evolved stars. Sufficient material is available to nourish a black hole in each nucleus, and they have many characteristics expected of a dust-enshrouded quasar⁶.

The first powerful extragalactic OH maser was discovered in Arp 220 in 1982, and it became the prototype of the class of so-called megamasers, because its luminosity exceeds that of galactic masers by at least four orders of magnitude⁷. Early low-resolution observations of the megamaser indicated that its emission mimicked that of the continuum emission, suggesting that the maser originated in a large-scale envelope that amplified the continuum emission.

About 50 other megamasers have been identified with distances up to about 500 megaparsecs (ref. 8). The luminosity of these masers increases as the square of the infrared luminosity, which can be understood if the masers are low-gain amplifiers of the background continuum radio source, as the pump power to the maser and radio luminosity of the amplified source are both proportional to the infrared luminosity⁹. Compelling as this model seems, no direct evidence of the precise alignment of the masers and their background sources has been found until now.

Lonsdale *et al.* were studying the structure of the compact radio synchrotron emission from the nuclei of Arp 220 with an intercontinental array of telescopes to obtain images with a resolution of about 3 milliarcsec. They inadvertently and fortuitously set their frequency bands to cover the emission from the megamaser at 18 cm wavelength. What they discovered (in an analysis concentrated on one of the nuclei) was very compact maser emission exactly superimposed, to the limit of their measurement accuracy of about 0.2 parsec, on a compact component of the nuclear continuum emission. Furthermore, they found that the masing components, which spanned a velocity range of about 400 km s⁻¹, were all coincident to within a few parsecs.

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RIBOZYMES

Time to change partners

Tan Inoue

They argue that the probability of chance alignment would be small if the masers were in a cloud considerably distant from the nucleus, because the maser emission would become highly beamed and the probability that we would see it would become small. Therefore they conclude that the maser emission most probably originates within a volume of radius 10 parsec centred on the nucleus.

The picture that emerges is necessarily sketchy, given the very limited nature of the observations. But the prospects for future observations from more comprehensive experiments are tremendously exciting. If the masers arise in a molecular torus, with a keplerian velocity of 200 km s^{-1} (corresponding to the range of maser emission), surrounding a black hole of 10^7 times the mass of the Sun, then this torus should be readily apparent in the velocity distribution of maser emission.

The authors are mounting a large-scale VLBI experiment involving 15 stations, including the recently completed Very Long Baseline Array and the telescopes of the European VLBI Network, in order to make a detailed image of the maser. This will allow them to study the amplification model in detail and determine the exact kinematic structure of the nuclear regions on a scale of 1 parsec. The proper motions of the maser spots would be expected to be about 0.5 microarcsecond per year, which, unfortunately, would be too difficult to measure with present ground-based instruments.

A look at the subparsec level structure will be possible with the advent of satellite-borne VLBI telescopes scheduled for launch in 1997 from Japan and Russia. The best chance of measuring proper motions in such a nuclear toroid may be afforded by observations of a cousin of the OH megamaser, the water vapour megamaser, which emits at a wavelength 15 times shorter than OH and therefore can be observed with a resolution 15 times finer. Such masers have been found in about a dozen bright infrared galaxies but not, alas, in Arp 220. □

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RIBOZYMES — RNAs with enzymatic activity — can catalyse chemical reactions in the absence of protein, but in many instances known ribozymes are associated *in vivo* with proteins that enable the ribozyme to perform its task efficiently. According to the 'RNA world' hypothesis, it is generally believed that, in the course of evolution, these proteins would have gradually taken over from RNA structural elements in the ribozyme itself. But the question has always been "Is there any evidence?". Now, on page 147 of this issue, Mohr *et al.*¹ provide this evidence, by showing that a protein associated with a ribozyme from the mould *Neurospora* can replace a functional non-catalytic RNA domain in the *Tetrahymena* ribozyme.

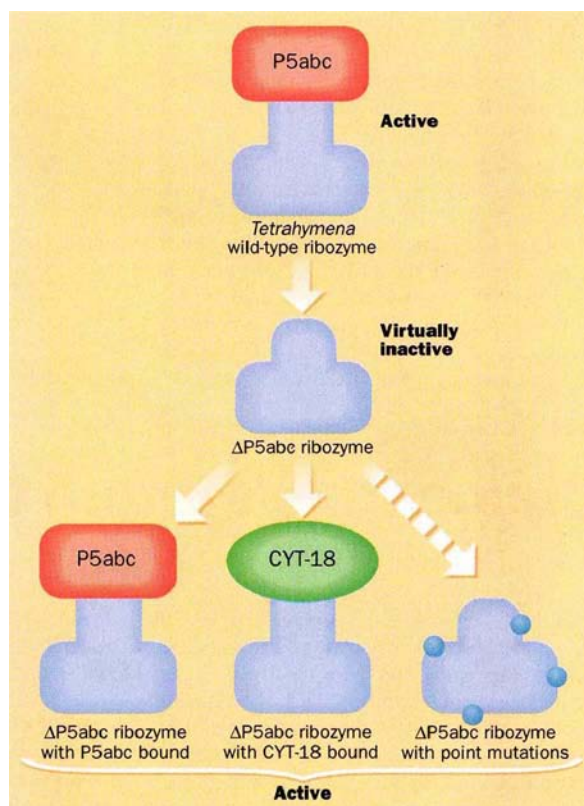
The *Tetrahymena* LSU ribozyme is a group I intron, in the precursor 26S rRNA, which splices itself out of the precursor during rRNA maturation². More than 200 group I introns have been sequenced and all can be folded into a similar general structure³. In the *Tetrahymena* ribozyme, however, there is a large extra portion called P5abc, which forms an extension to the core secondary structure.

Ribozymes lacking the P5abc extension (Δ P5abc) can still perform the self-splicing reaction accurately, but very much more slowly than the wild-type ribozyme. The function of P5abc must therefore be to enhance enzyme activity; indeed, if P5abc is prepared separately and added back to Δ P5abc, the splicing reaction is accelerated tremendously⁴. Binding of P5abc to the catalytic core of the ribozyme through tertiary interactions transforms the core structure into the active form.

In *Neurospora*, the CYT-18 protein — the mitochondrial tyrosyl-tRNA synthetase — seems to perform a similar function to P5abc, binding to *Neurospora* and other group I introns to promote RNA splicing⁵. Could protein CYT-18 therefore substitute for P5abc RNA in the *Tetrahymena* splicing reaction?

Mohr *et al.* designed an experiment to answer this question. They incubated CYT-18 protein with an inactive *Tetra-*

hymena ribozyme mutant lacking P5abc (Δ P5abc) to see whether it could restore splicing activity. The answer was an unequivocal yes. As would be anticipated if the protein is substituting for the P5abc structure, CYT-18 was incapable of binding to or activating the intact wild-type ribozyme containing P5abc.



Activity of various derivatives of the *Tetrahymena* group I intron. The wild-type ribozyme functions as an active enzyme. Removal of the P5abc extension, producing Δ P5abc, reduces activity drastically. Addition of the CYT-18 protein restores activity, as does adding back the P5abc RNA. By analogy to other systems, activity could also be restored by point mutations in Δ P5abc.

Structural analysis of the newly constituted protein-ribozyme system reveals that (1) the CYT-18 protein and the P5abc RNA bind to overlapping regions on the ribozyme, and (2) the core structure of the ribozyme presumably transforms into the same active form when either CYT-18 or P5abc is bound. The protein and the RNA apparently activate the ribozyme in the same manner. But there is still much to be elucidated. Although CYT-18 binding to the ribozyme is substantially stronger than for P5abc RNA, the efficiency of protein-assisted splicing is more than a hundred times less than that of the RNA-assisted reaction. We still need to know where (and how) the protein and the RNA bind, and how (and why) the ribozyme structure

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is stabilized to promote splicing.

By progressively losing self-splicing capability and increasing their dependence on *trans*-acting proteins and snRNAs, ribozymes such as group I and group II introns presumably evolved into nuclear mRNA introns and spliceosomes^{6,7}. We now have intriguing evidence to support this hypothesis.

Then what were ribozymes like before they gained these auxiliary units — whether integral or *trans*-acting? One feasible answer is that efficient RNA-catalysed reactions should have been performed by ribozymes lacking auxiliary units. This is indeed the likely answer. A mutant group I intron in the bacteriophage T4 *sunY* gene that lacks all extra structures is inactive like the $\Delta P5abc$ *Tetrahymena* ribozyme. But by introducing point mutations into this mutant gene, a stable and active RNA structure could be obtained. One mutant, with five point mutations, had extremely high catalytic activity, even compared with wild type⁸. A group I intron RNA lacking any extra structural elements or association with *trans*-acting proteins can thus perform

highly efficiently. So far, the research outcomes are consistent with the evolution hypothesis.

The implications of the CYT-18 protein-ribozyme system are ingenious and pleasing. Many RNA-protein complexes other than ribozymes or spliceosomes mediate gene expression or its regulation. Many workers are now investigating these systems⁹, and it will be interesting to see whether the splicing systems and other systems share any distinctive features. □

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LUNAR SCIENCE

Clementine's return to the Moon

Clark R. Chapman

TWENTY-FIVE years ago this month, on 20 July 1969, Neil Armstrong made his first small step onto the Moon. Only three years later, in December 1972, the Apollo programme ended prematurely, leaving behind several scientific ALSEP stations (Apollo Lunar Surface Experiment Packages). In 1976, the Soviet Luna 24 mission returned lunar samples from Mare Crisium. The following year, to save a mere \$200,000 per year, NASA turned off the five functioning ALSEP stations, ending scientific exploration of the Moon for a decade and a half.

Now we are back to the Moon, thanks to the Clementine mission, a highly promoted new way of doing planetary exploration using simple spacecraft and cheap operations. Earlier this decade, the Galileo spacecraft made two passes through the Earth-Moon system *en route* to Jupiter, obtaining multispectral data for parts of the lunar far side and north pole. But Clementine's two-and-a-half month mission, completed just two months before the twenty-fifth anniversary of the first moonwalk, acquired a global database that will give new life to lunar science. As an example of how to revitalize planetary exploration generally, Clementine's model is more cloudy.

Clementine's day in the limelight came on Thursday 26 May, during a special

session of the American Geophysical Union meeting in Baltimore*. Although few scientific results were ready just weeks after the end of the brief mission, dramatic examples of Clementine's global database were presented, along with preliminary analysis. Also on the programme was a paper by the scientific father of Clementine, Lowell Observatory geologist Eugene Shoemaker, who was to describe the would-be encounter with near-Earth asteroid Geographos and a possibility for using reserve propellant to go beyond to a second asteroid. But a few weeks before the AGU meeting, still unexplained computer malfunctions sent Clementine into a wild spin and the transfer trajectory to Geographos was cancelled. The spacecraft is now back in Earth orbit, crippled as an interplanetary probe, and Shoemaker instead waxed eloquent about the Moon.

The lunar science data were impressive. Back in the 1960s, a series of Lunar Orbiters had obtained photographic global coverage of the Moon on long strips of film. Adequate for planning the Apollo landings, the pictures were not photometric and they have been little used for quantitative lunar research. Subsequent Apollo remote-sensing of the Moon was

restricted to groundtracks along Apollo orbits, leaving large portions of the Moon (including much of the far side) terra incognita.

Clementine has changed all that. Systematic digital mapping, by several cameras in wavelengths from the ultraviolet to the thermal infrared, has covered 99.9 per cent of the Moon. Talks by C. Pieters (Brown Univ.) and A. McEwen (US Geological Survey) left little doubt that quantitative multispectral maps can be derived from the data that will provide the first comprehensive, global picture of how the Moon's heterogeneous mineralogy is related to its geological structure.

The big scientific bonanza from Clementine revealed in Baltimore came from laser altimetry. This showed that the Moon's range of topographic elevation varies by nearly twice the amount previously suspected. The South Pole-Aitken basin on the far side was not even widely recognized and accepted until the mid-1970s; more recently, it was spectrally mapped from afar by Galileo during its first Earth-Moon encounter. But nobody was prepared for Clementine's result: the basin turns out to be 12 kilometres deep, making it the largest, deepest crater in the Solar System (P. Spudis, Lunar and Planetary Institute, Houston). Also, what had been thought to be a minor basin visible from Earth (named Mendel-Rydberg) turns out to be 5 km deep. The fundamental structure of the lunar crust, which seems so dissimilar between the front and back of the Moon, is now revealed — according to Shoemaker — to be the superposition of repeated impacts during the epoch of early bombardment. Its thickness varies from about 10 km under the South Pole-Aitken basin to more than 100 km elsewhere.

Preliminary interpretations of Clementine's new lunar gravity map can already be made and the Bouguer anomalies calculated from the associated topography (Maria Zuber, Johns Hopkins Univ.). Clementine has revealed geophysical complexity far beyond the 'masscons' (mass concentrations associated with lunar basins filled with dense lava flows) that were so much discussed a quarter of a century ago. Although the Orientale basin has been found to have complex, ring-like gravity anomalies, Mendel-Rydberg seems to be fully compensated. These are just the tip of the iceberg of scientific insights promised by the Clementine database.

Clementine has been controversial from its start, a few short years ago. It was developed by the SDIO/BMDO ('Star Wars') office of the American defence department as part of a 'long duration space test' of its military hardware, which then invited NASA to add scientific advisors. Although there had been doubts that the data would be good enough to

*1994 Spring Meeting of the American Geophysical Union, Baltimore, Maryland, 23–27 May 1994.

be scientifically useful, no sceptics were left after the Baltimore meeting. But Clementine's faster-smaller-cheaper approach to planetary exploration was tarnished, despite the lunar success. After all, failure less than four months after launch does not make Clementine auspicious as a prototype for future interplanetary spacecraft.

The Jet Propulsion Laboratory, in its attempt to 're-invent' itself and adapt to budgetary pressures for cheaper missions, had sent its high-level managers to study Clementine's 'Batcave' operations centre in suburban Washington DC. Although the full costs of Clementine may never be known, as it was developed in secrecy, JPL and NASA had felt challenged to consider Clementine as a paradigm for its future missions. Some NASA managers had even complained that Clementine's SDIO promoters were overselling their mission and putting NASA's own new thrust for cheaper missions (the Discovery Program) in a bad light.

Now, we must understand what attri-

butes of Clementine led to its lunar success, as well as whatever led to its subsequent failure. What elements of Clementine's operation are technically and fiscally sound models for future missions, at acceptable levels of risk? What must be changed for missions whose primary goals are scientific exploration rather than testing defence hardware?

Speaking in advance of his appearance at the Clementine press conference in Baltimore, NASA's chief of Space Science and Applications, Wesley Huntress, said that he was unaware of any plans by NASA or any other governmental agency to perform a comprehensive analysis of the Clementine experiment from the standpoint of its lessons for civilian space science. Clearly, if Clementine is to provide guidance for the next quarter century of international lunar and planetary exploration, such a study must be undertaken, and soon. □

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Standard Model, it should be accompanied by a heavier partner quark, just as the up and down, and charm and strange, quarks form pairs. Without the top quark, the edifice of the Standard Model would have come tumbling down. However, it does not complete the Standard Model; the origin of the masses of the quarks, leptons and intermediate vector bosons W and Z remains to be understood, which is where the Higgs boson would come in.

The evidence for the top quark reported from CDF consists of a dozen or so events containing either two W bosons (seen via their decays into an electron or muon and a neutrino) or one such W together with three or four jets of hadrons. These are believed to be due to the production of a top quark-antiquark pair, with both subsequently decaying into an identified W and a bottom quark or antiquark, or one top decaying this way and the other into another bottom and quark-antiquark pair. Boring old quantum chromodynamics (QCD), the fairly well understood theory of the strong nuclear interactions, should also produce a few events with jets accompanying a W boson. But some of the CDF events have distinctive top-antitop features: for example, many of them contain bottom quarks or antiquarks, and their kinematic characteristics do not look very QCD-like. Therefore the very complete and well documented analysis by the CDF collaboration, in both the present paper² and the longer one still in press³, persuades most particle physicists that the top quark has indeed been found.

That the Fermilab proton-antiproton collider produced any top quarks at all is a tribute to the skill of their accelerator engineers, and the careful analysis is a tribute to the CDF collaboration. It is disappointing that the rival D0 collaboration cannot yet confirm the evidence, but their lack of a signal is generally ascribed to bad luck.

The CDF evidence is not yet conclusive, but has been accepted readily by the particle physics community — perhaps in part because the announced mass of the top quark, 174 ± 16 GeV (see Fig. 1), agrees very well with theoretical predictions based on precision electroweak data from the Large Electron-Positron Collider (LEP) and elsewhere. These measurements are sensitive to the quantum corrections due to virtual particles, such as the top quark or Higgs boson, that fluctuate out of the vacuum for very small periods of time limited by the uncertainty principle. Measurements of these small quantum corrections allow us to set limits on the masses and other properties of the heavy particles, even if they cannot be produced directly.

At LEP, the mass and many decay properties of the Z boson have been measured with high precision, enabling

HIGH-ENERGY PHYSICS

On top of the particle world

John Ellis

FOLLOWING its highly publicized announcement in April this year (see ref. 1), evidence for the discovery of the top quark is published this week² by the Collider Detector Facility (CDF) collaboration, working at the proton-antiproton collider at the Fermi National Accelerator Laboratory (Fermilab) in the United States. After more than a year of optimistic excitement and pessimistic caution flashing over the Internet rumour mill, the evidence is available for evaluation by high-energy physicists.

It seems, then, that the penultimate building block of the Standard Model of particle physics is finally in place. The news sharpens the interest in the search for the last building block of the Standard Model, the mythical Higgs boson, and improves indications of its mass. The high mass of the top quark apparently measured is in line with some suggested extensions of the Standard Model based on supersymmetry, but raises some problems for alternative suggestions that the Higgs boson might actually be a composite field.

According to the Standard Model, all strongly interacting

nuclear matter is made out of six fundamental quark constituents. Five types of quark — up, down, strange, charm and bottom — have been well established for some time. The bottom quark was the heaviest previously known, weighing about 5 GeV, and also the most recently discovered, in 1977. According to the

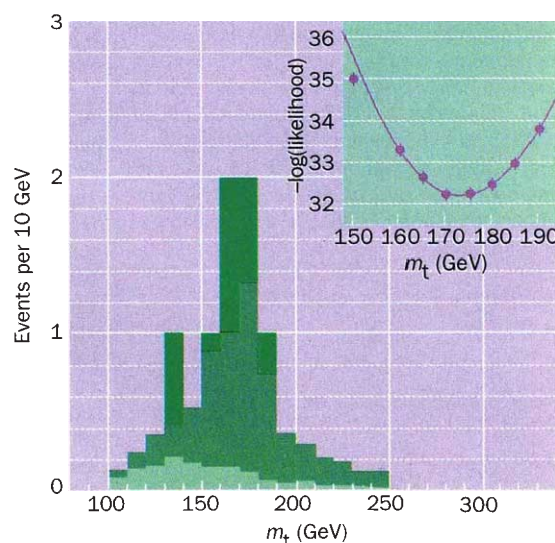


FIG. 1 The central value of m_t extracted by CDF from seven of their events (dark green histogram) is about 174 GeV (see inset). The systematic and statistical errors combined are about 16 GeV. The pale and palest green histograms are from Monte Carlo calculations of the expected top signal and QCD background respectively^{2,3}.

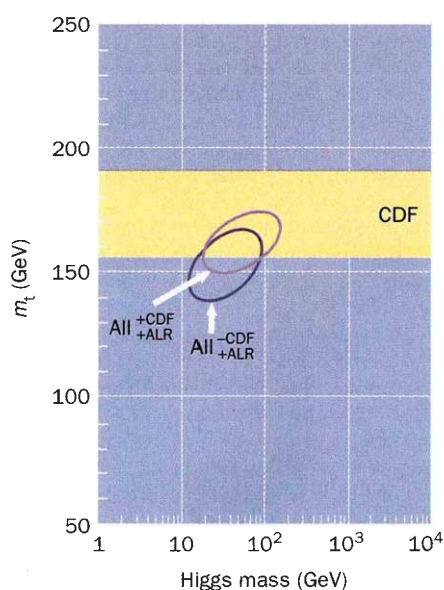


FIG. 2 A global fit (All) to precision electroweak data from LEP⁴ and elsewhere, including the recent SLAC measurement of the left-right polarization asymmetry ALR⁵, gives the $\Delta\chi^2 = 1$ contour indicated by All^{+CDF}_{+ALR} (ref. 6). The band labelled CDF is the ± 1 standard deviation range given in refs 2 and 3. The $\Delta\chi^2 = 1$ contour labelled All^{+CDF}_{+ALR} combines CDF with the precision electroweak data⁶.

the top quark to be estimated⁴ as 171 ± 15 GeV. Previous lower-energy experiments gave a rather lower estimate whereas a recent measurement⁵ at the Stanford Linear Accelerator Center (SLAC) of the different rates for production of Z bosons with different electron beam polarizations tends to increase it. One global fit⁶ to all the available collider and precision electroweak data now gives the mass as $m_t = 162 \pm 9$ GeV, as shown in Fig. 2.

The striking agreement between the CDF measurements and the theoretical prediction derived from LEP and other precision data is an impressive success for the Standard Model. It provides confirmation of the theory at the quantum level, comparable to the confirmation of quantum electrodynamics by measurement of the Lamb shift. This continues a theoretical tradition of successfully predicting the masses of heavy particles in the Standard Model. The mass of the charm quark was predicted from an analysis of rare strange particle decays⁷, the bottom quark mass was predicted⁸ on the basis of Grand Unified Theories, using the tau lepton mass as input, and the W and Z masses were predicted on the basis of the observed strength of the weak interactions at low energies.

The observed top mass may provide us with some clues to what lies beyond the Standard Model. It fits with some speculations in the context of supersymmetry, according to which a heavy top quark triggers the spontaneous breakdown of electroweak symmetry that permits the

Higgs boson to give masses to all the particles in the Standard Model. Such a heavy top quark is also natural in many string models, which require at least one quark to be heavier than the W and Z bosons. However, the observed top quark mass is too small for many models that wanted to replace an elementary Higgs boson by a composite state made out of top quarks and antiquarks, analogous to the Cooper pairs of superconductivity.

What next? Clearly CDF (and, we hope, D0) should accumulate more candidate top events, and one new event from their current data-taking run is already rumbling around the Internet. However, CDF do not expect to have more than a few hundred top quarks before the end of the century. In the following decade, CERN's Large Hadron Collider (LHC) will, we hope, be able to produce millions of top quarks, making it possible to measure the mass much more precisely and to search for rare decay modes, perhaps into charged Higgs bosons.

The experimental focus now sharpens on searches for the Higgs boson, or whatever replaces it as the source of particle masses. Even before the CDF top quark report, a combination of LEP and other precision electroweak data indicated⁴ that the Higgs boson might not be very heavy. The uncertainty in this estimate can now be reduced, and a Higgs mass of around 100 GeV — as predicted in supersymmetric models — seems to be favoured⁶. The allowed range of Higgs masses is still large, but I personally would give 2-to-1 odds that it weighs less than 300 GeV, on the basis of the present data.

Fermilab's proton-antiproton collider is not favoured to find the Higgs. For the rest of this decade, most eyes will be on LEP, where the planned energy upgrade should enable a Higgs boson to be found if it weighs less than about 90 GeV. Beyond that, the LHC should eventually be able to explore all the mass range up to about 1,000 GeV. The hope is that the discovery of a Higgs boson will finally take us beyond the Standard Model, into a new world. My hunch is that it will be supersymmetric. □

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See the smell!

SMELL is a wonderfully evocative sense, but maddeningly vague. Our two nostrils fail to provide a good stereo image, or even to indicate where a smell comes from. Daedalus plans to assist them.

All smelly substances are, of course, volatile. Daedalus reckons that heat radiation can evaporate them by a quantum effect. On hitting a volatile surface, a photon with exactly the right energy of evaporation should be perfectly absorbed, evaporating one molecule in the process. As with the related photoelectric effect, the exchange should be highly efficient. The surface should absorb the photons totally, and appear completely black at that wavelength. Adjacent wavelengths, however, would reflect normally. Daedalus calculates that most substances should have their 'resonant evaporation' frequency somewhere in the near infrared.

So DREADCO engineers are working on a novel 'smell camera'. Its infrared diode laser scans a narrow raster beam over the scene to be imaged. The laser is modulated rapidly between the resonant evaporation frequency of the target substance and a nearby passive reference frequency. A surface containing that substance will absorb the resonant radiation while reflecting the reference beam: it will return a strongly amplitude-modulated signal. Other surfaces will reflect both frequencies about equally and will return no modulation. On the display, therefore, regions containing the target substance will show up brightly on a dark background. As the smell camera detects the evaporation itself, not merely a few of the resulting vapour molecules, it will be amazingly sensitive.

The smell camera will find many household uses. The spot on the carpet where the cat has disgraced itself, the dry rot in the corner of the ceiling, the area of redecoration where the paint is not yet dry, the neglected kipper in the pantry, all will spring clearly into view. Industrialists will use it to detect spillages, leaks and the origins of escaping odours. Large versions flown in aircraft will scan the countryside or industrial estates for specific pollutants, and identify slicks at sea. Biologists will use it to study the sex pheromones emitted by female moths and animals on heat, and the pattern of scent release in flowering plants.

In airline security, the smell camera will be a powerful rival to the sniffer dog. Not only will it scan endless items of luggage for traces of explosives and drugs, never tiring or wandering; it will scrutinize their owners for suspicious signs of nervous sweating. David Jones

Subliminal perception on TV

SIR — Can we be influenced by subliminal messages while watching television? Messages below the threshold for awareness can be effective under laboratory conditions^{1,2}, but little is known about the effects of broadcast messages. We have taken advantage of a rare opportunity to observe the effects of a subliminal message on a large scale which was recently presented by BBC television's "Tomorrow's World" programme on 25 March 1994. The purpose of our study was to determine whether a simple stimulus — a picture of a woman's face, smiling — could influence the judgement of emotion in a subsequently presented picture of an expressionless face.

Our immediate responses to simple items such as single words and line drawings can be influenced if these subliminal priming stimuli are presented below a threshold for awareness individually determined for each participant^{3,4}. One problem with subliminal messages in radio and television broadcasts is that they must be presented at a fixed signal-to-noise ratio, whereas individuals vary in their thresholds for reportability. What would be a subliminal message for one individual would reach awareness for another, and doubts have been expressed about the possible commercial effects of messages with fixed signal-to-noise ratios⁵⁻⁷.

In the TV broadcast, the subliminal face was presented during a 25-second film of two infants playing with an adult. The face was superimposed over one frame of the film, and was present for 20 milliseconds on each of seven presentations. The eastern network regions of the BBC received the film with these subliminal frames, and the western regions received the same film but without the frames. Immediately after the film, television viewers were invited to make a judgement about a face that expressed no emotion. Viewers in the western regions provided the baseline judgement on the facial expression. The judgements were made by telephoning one of two numbers — one to indicate a perception of happiness, and one for sadness. Up to the point in the programme when the results were announced, 34,327 telephone calls were received and overall the total was 37,437 calls. The distribution of judgements between regional groups (subliminal frames and controls), and between the perception of happy and sad, are presented in the table.

The observed frequencies given in the table were compared with a chi-square test, finding $\chi^2 = 9.85$ ($P < 0.01$) for the immediate calls and $\chi^2 = 12.32$ ($P < 0.001$) if all calls were included. The viewers who received the subliminal smiling face were less likely to judge a neutral face as being happy than were those viewers

who saw no subliminal frames.

We should emphasize that the direction of the effect is not what was expected on the basis of previous laboratory studies of facial judgements preceded by subliminal words^{1,2}. The direction of the effect calls for explanation. One possibility is that for some viewers the frames were not subliminal, and that their judgements of sadness were based upon an explicit comparison between the expression on the

RESULTS FROM TV BROADCAST AND LABORATORY STUDIES

TV broadcast*	Judged "happy"	Judged "sad"
Subliminal frame	9,606 (10,403)	7,790 (8,544)
No subliminal frames	9,634 (10,654)	7,297 (8,136)
Laboratory		
Informed group	10	42
Uninformed group	24	24

In the TV broadcast study the figures give the number of telephone calls made from two network regions (subliminal frames, and no subliminal frames) judging a face to be happy or sad (figures in parentheses are totals, including calls received in the period immediately after the announcement of the results). In the laboratory study, figures represent the numbers of adults judging the neutral face to be happy or sad, according to whether they knew that subliminal frames were present or not.

*J. Bunting and D. Purvis of BBC television's "Tomorrow's World" designed and produced the video tapes and P. Niedenthal, N. Dixon, A. Greenwald, J. Kihlstrom, T. Moore and R. N. Sykes helped with the investigation.

face at one moment (during the film) against how the woman appeared later. During the film the face was happy and later it was expressionless, suggesting that the woman had become sadder. This contrast explanation is consistent with the idea of early stimuli generating an adaptation level⁸, against which later stimuli are compared, and has been used to account for changes in judgements of emotion in sequences of faces⁹.

A second explanation of the effect is that it is an artefact of regional differences in the perception of emotion in faces and that even without any subliminal stimuli, the present pattern of results would have been obtained. We have no evidence for or against this explanation.

The broadcast study provides no information as to whether the frames were available to awareness or not. To determine whether the frames were subliminal for viewers similar to those likely to be watching the television programme, two laboratory groups were tested before the

broadcast programme. Groups of adults of a wide variety of ages and backgrounds were asked to give written reports on the frames, and then to make a judgement on the expressionless face. One group ($N = 48$) was not informed in advance of the presence of the frames (they were informed immediately after they viewed the film and had made a happy/sad judgement), and the other group ($N = 52$) was informed and instructed to look specifically for the subliminal frames. No viewer in the informed group reported seeing the smiling face, and only one viewer in the uninformed group reported seeing a face. There is little evidence here to suggest that the frames were reportable, but the judgements of expression did show some differences, and these are also presented in the table.

These observed frequencies were also compared with a chi-square test, giving $\chi^2 = 10.53$ ($P < 0.01$). Knowing that the subliminal frames were present did not increase the reportability of the smiling face, but it did result in a tendency to see the neutral face as being sad. This is the same direction of effect as seen in the broadcast study, a contrast effect. We must point out that even if the effect in the broadcast study was a consequence of the subliminal perception of the face, it was a very small effect. The effect may have resulted from a regional bias in the categorization of facial emotion, however; also, we cannot eliminate the possibility that the frames containing a smiling face were above the threshold for awareness for a small number of participants.

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Scientific Correspondence

Scientific Correspondence is a relatively informal section of *Nature* in which matters of general scientific interest, not necessarily those arising from papers appearing in *Nature*, are published. Because there is space to print only a small proportion of the letters received, priority is usually given according to general interest and topicality, to contributions of fewer than 500 words, and to contributions using simple language.

Phototactic purple bacteria

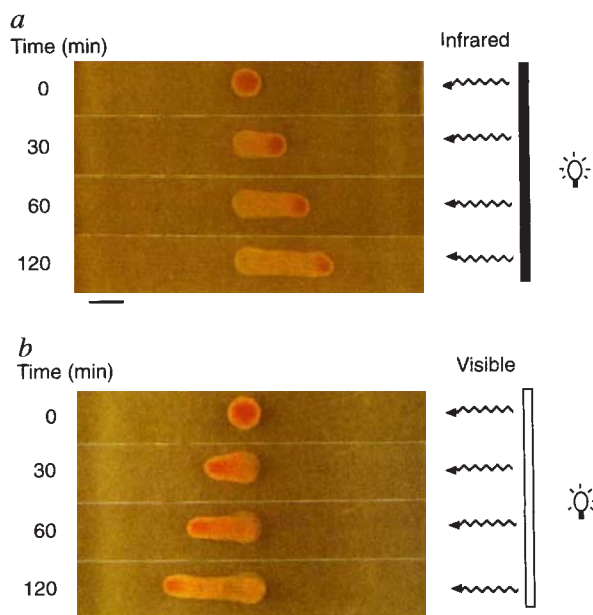
SIR — In 1883 T. Engelmann¹ discovered that purple photosynthetic bacteria reverse their swimming direction when the light intensity is suddenly reduced. He described this photosensory effect as "Schreckbewegung" ('movement of fright'), but it is more properly designated as a scotophobic response, that is, a fear of darkness. Scotophobic behaviour is typical of motile purple bacteria, is independent of the direction of illumination, and does not occur if the light intensity is reduced only gradually. By contrast, oxygenic cyanobacteria and algae exhibit phototactic responses, that is, oriented movement towards or away from a light source, irrespective of its intensity. We show here that the non-sulphur purple bacterium *Rhodospirillum centenum*² can also move with respect to the direction of illumination and that this phototactic behaviour is observable macroscopically.

When grown phototrophically in a liquid medium, *R. centenum* cells bear a single polar flagellum, but when grown aerobically in darkness on 0.8% agar containing 0.3% peptone they produce many peritrichous flagella. Such cells contain the normal complement of photosynthetic pigments³ and form phototactic 'swarm colonies': the figure shows that lateral illumination of such a colony with infrared light causes positive phototaxis (a) or negative phototaxis with visible light (b). Action spectra and details of the responses of swarm colonies to simultaneous illumination with perpendicular beams of light will be published elsewhere (manuscript in preparation).

Several *ad hoc* assumptions would be necessary to explain the movement of *R. centenum* swarm colonies towards or away from light sources on the basis of scotophobic responses of randomly moving cells. Nevertheless, we also tested for authentic phototaxis using a technique previously applied to phototactic algae⁴. Infrared light was passed through a converging lens to focus on a swarm colony. At the edge of the colony, the light intensity was relatively high and the intensity gradient decreased in the direction of the light source. A positive phototactic organism or colony should move towards

the light source through the gradient of decreasing light intensity; on the other hand, if their movement is scotophobic, the colony should move in the opposite direction. *R. centenum* swarm colonies irradiated laterally with infrared light in fact moved rapidly towards the light source, as for positive phototaxis.

Anoxygenic photosynthetic bacteria and the oxygenic cyanobacteria and algae share several similar physiological requirements, so it is not surprising that



Phototactic behaviour of *R. centenum* swarm colonies by time-lapse photography. a, An *R. centenum* swarm colony exposed to 575 μ W of infrared light of wavelength above 650 nm; b, swarm colony exposed to 2,500 μ W of visible light of wavelength below 650 nm. Scale bar, 10 mm.

representatives of these three groups of phototrophs are phototactic. Phototaxis is believed to help motile photosynthetic organisms move towards zones where conditions are better for growth; for example, it is well known that cyanobacteria and anoxygenic photosynthetic bacteria accumulate in discrete bands in stratified lakes and laminated microbial mats⁵.

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Extinction or miscalculation?

SIR — Heywood *et al.*¹ have discussed uncertainties in modelling rates. One way to check a predictive model's validity is to see how well it predicts past events. In this case, the species–area relation model does fairly well in predicting the number of bird and mammal extinctions during the past 500 years in the United States and Canada.

Here I consider species extirpated following habitat destruction initiated by Europeans in North America², combined with currently endangered or threatened species³, to check if species loss is congruent with habitat loss. Although exact figures are difficult to come by, I take as 20% the amount of land removed from native North American mammal and bird habitats⁴. Applying the species–area relation, $S_0 = kA^z$, gives the number of species originally present, and $S_1 = k(0.8A)^z$ gives the number remaining today (for non-isolates $0.12 \leq z \leq 0.17$; for true island isolates $z \approx 0.25$; ref. 5). Thus $1 - (S_1/S_0) = 1 - (0.8)^z$ is the fraction of original species lost.

The values of z usually observed for non-isolates indicate species loss is in the range 2.65–3.72%. The number of terrestrial bird species threatened, endangered or extinct in North America is 18. The question is, then, does 18 correspond to 2.65–3.72% of the original bird fauna? Working backwards to deduce T , the total number of bird species originally present, gives $679 \leq T \leq 484$, which does bound the roughly 600 known breeding species in North America, plus extinctions.

I used the non-isolate z because birds are good dispersers and 'habitat islands' are less likely to be truly isolated for birds than for mammals. For mammals, which do not disperse as readily as birds, I chose an isolate value for z of 0.25 (ref. 5). This indicates a species loss of 5.4% for mammals. Using 19, the number of terrestrial mammal species threatened, endangered or extinct in North America, and again working backwards, the original number of mammal species is around 352. This is in reasonable agreement with the roughly 300 extant species plus extinctions.

This thumbnail analysis supports the use of species–area curves to predict species extinctions due to habitat loss, at least

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for mammal and bird species. It also suggests that z values may be taxon-specific when dealing with continental biotas.

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SIR — Heywood *et al.*¹ propose an explanation for the discrepancy between the high extinction rates predicted by theory and the low rates observed in reality. The admission that there is a discrepancy is something of a breakthrough. Theoretical predictions of extinction rates, derived from species–area relations, have previously been presented as if they were verified facts^{2–4}. Indeed, the popular press, environmental organizations and many politicians have taken them to be such; witness the frequently cited ‘fact’ that 50,000 species a year are being driven to extinction, or a statement to the UN commission on sustainable development by US vice-president Al Gore that one-half of all species may be extinct within the lifetime of our grandchildren.

Heywood *et al.* seek to reconcile the discrepancy by arguing that there is a time-lag of some indeterminate length during which species that are ‘committed’ to extinction persist. An equally parsimonious explanation is that the predictions are simply wrong. In an earlier publication, two of the co-authors of ref. 1 argued that the species–area relation is a poor tool for making predictions: in a mainland situation, it is “nothing more than a self-evident fact: that as one enlarges an area, it comes to eventually encompass the geographical ranges of more species. The danger comes when this is extrapolated backwards, and it is assumed that by reducing the size of a forest, it will lose species according to the same gradient.” Yet that is precisely what was done to obtain the oft-cited, very high extinction-rate figures. Wilson² uses the species–area relation $S = kA^z$, and, plugging in an estimate of the rate at which tropical forests are being cleared and using $z = 0.15–0.35$, calculates that “if deforestation continues for thirty more years at the present rate, one-tenth to one-quarter of the rain-forest species will disappear”.

As Heywood and Stuart⁵ pointed out, there are many reasons why the species–area relation may be descriptive without being predictive, especially on a global basis: species are not distributed at random; conservation measures already protect many key habitats (including those critical to 95 per cent of Afrotropical avifauna, for example); some species may be able to adapt to secondary habitats. In an earlier review of the species–area relationship, Connor and McCoy⁶ noted that the power relationship $S = kA^z$, first pro-

posed in 1921, tends to exaggerate, producing impossibly high values of species when extrapolated to large areas. The authors’ analysis of 100 sets of species–area data led to doubt that there is any biological significance to the relation whatsoever; they suggested it may be nothing more than a sampling phenomenon, a “correlation... without a functional relationship”. Far from being proof that some consistent biological law was in operation, the fact that the values of z (determined by the slope of a best linear fit of $\log S$ versus $\log A$) typically fell in the range 0.2–0.4 was, they found, simply characteristic of any regression system with a high r -value and a large range in the independent variable relative to that of a monotonic dependent variable.

Heywood *et al.* take comfort from one quasi-empirical test of the theoretical extinction rates calculated from the species–area curve: A species-by-species analysis of threatened birds tallied 450 that appear doomed to extinction, a mere factor of 3 (as opposed to 100) off from the predicted value of 1,350. Yet Heywood and Stuart noted that hunting, collecting, disease and introduced species (as opposed to loss of habitat area, the presumed driving force in the theoretical predictions) have had “very major effects” on the decline of these particular species, most of which are not associated with tropical forests in any event. It is the loss of tropical forests that drives the very high global extinction-rate predictions, however. And even allowing for the operation of time lags, large losses in tropical forest area have occurred at least in one case without any comparable loss in species. The Atlantic coastal forests of Brazil, which have a higher species diversity than the now-threatened Amazon forests, were reduced by nearly 90 per cent, mostly in the nineteenth century; a recent survey by zoologists could not document a single extinction. On the contrary, several birds and six butterfly species believed 20 years ago to be extinct were rediscovered⁷. According to the species–area relationship, a loss of 50 per cent of species should have occurred. Given such discrepancies, one would appear to be justified in continuing to take the much-cited global extinction rates with a grain of salt.

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Self-assembly and protein stability

SIR — Shen *et al.* report¹ a dramatic increase (to 140 °C) in the thermal stability of bacteriorhodopsin in dry films of purple membrane. The potential high-temperature applications of these dry films were emphasized in an accompanying News and Views article². In general, thermostabilization is often required (or desirable) for technological applications of proteins, and the results of Shen *et al.* suggest that removal of water may be a convenient way to achieve this goal.

In fact, protein thermostabilization on dehydration is not without precedents. In several cases, dry or lyophilized enzymes have been shown to be fairly resistant to thermal inactivation, even at temperatures above 100 °C; for instance, the half-lives for the irreversible thermal inactivation of dry trypsin and ribonuclease on heating *in vacuo* are about 2 h at 160 °C and about 10 min at 200 °C (calculated from ref. 3). Enhanced thermal stability is also observed when lyophilized enzymes are suspended in anhydrous organic solvents⁴, and the possibility of high-temperature catalysis is one of the features of nonaqueous enzymology (note that, even under such anhydrous conditions, a certain number of essential water molecules must remain bound to the protein^{4,5}).

Clearly, some applications of proteins may require a ‘solid-like’ support (such as a thin film); in these cases, immobilization of the protein in the suitable support (by, for instance, entrapment, covalent attachment, adsorption) followed by a controlled dehydration process appears to be an interesting possibility if thermostabilization is required.

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SAFINYA AND ROTHSCCHILD REPLY — We agree that there are several previously reported examples in which dehydration or suspension in anhydrous organic solvents leads to enhanced protein stabilization against denaturation. However in our study we observed stabilization of the two-dimensional order of the protein lattice in dry bacteriorhodopsin films, together with stability against denaturation at high temperatures.

It is now well established that individual two-dimensional bilayers of hydrated purple membrane exhibit a lattice-melting transition around 70 °C (ref. 1 and refs 23, 24 therein), and subsequently undergo a broad denaturing transition (from the

disordered phase) around 90–100 °C. In contrast, our work indicates that the stacking of these sheets (higher order self-assembly achieved by water removal) results in: (1) the complete suppression of the melting transition with the purple-membrane lattice remaining ordered; and (2) the absence of protein denaturation up to 140 °C. We believe that the high temperature stability at 140 °C is related to the retention of the two-dimensional ordered lattice which under hydrated conditions melts away around 70 °C. In other words the inter-protein interactions coming from the two-dimensional ordered lattice appear to prevent the protein denaturation.

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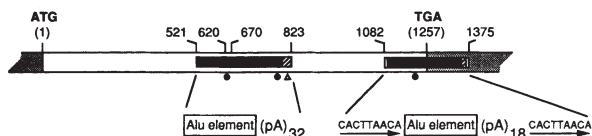
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Alu sequences in RMSA-1 protein?

SIR — Yeo *et al.*¹ report a previously undescribed chromosomal protein that “regulates mitotic spindle assembly”. The protein, termed RMSA-1, was identified as the antigen recognized by autoimmune serum from a patient with discoid lupus erythematosus. The antibodies in this serum bound to a protein of 47,000 (47K) on immunoblots of protein isolated from human, mouse, monkey and chicken cells. The serum was used to isolate a single human complementary DNA clone from a lambda zap cDNA expression library. The authors searched various sequence databases with the sequence of the putative RMSA-1 cDNA, but do not report the results of these searches in their paper.



The RMSA-1 open reading frame and untranslated regions. The open reading frame is shown as a large white box; 5' and 3' untranslated regions are grey; Alu elements are represented by black boxes; poly(A) tails by hatched boxes and these are shown in more detail underneath. The white boxes flanking the second Alu element are 9-bp target-site duplications (inset). The filled circles and the triangle are the putative p34^{cdc2} kinase sites and nuclear localization signal, respectively.

We searched the RMSA-1 DNA and protein sequences against the US National Center for Biotechnology Information's non-redundant nucleotide and protein sequence databases using the BLAST network service. Surprisingly, we found that the 418-codon open reading frame reported in this paper contains two Alu sequences (see figure). The presence of these Alu elements is absolutely unambiguous, whether protein or DNA sequence is used as query in the database search. The first Alu element runs from codons 173 to 278 of the putative RMSA-1 open reading frame, whereas the second begins at codon 364 and extends 112 nucleotides past the translational stop codon. The two Alu elements align with more than 70 per cent nucleotide identity with putatively active Alu elements², both contain characteristic poly(A) tails, and the second Alu element is flanked by a typical target site duplication. Of particular note, all three consensus p34^{cdc2} phosphorylation sites identified by Yeo *et al.* are contained within these Alu regions. Also, the poly-L-lysine sequence reported by the authors as a potential nuclear localization signal is encoded in the poly(A) tail of the first Alu element, which would contribute significantly to a predicted pI of greater than 10, rather than a pI of 6.33, as stated by Yeo *et al.*

Several issues are raised in the light of our observations. The presence of two translated Alu elements (accounting for 34 per cent of the encoded protein) calls into question whether the cloned cDNA encodes the 47K protein recognized on immunoblots, or whether, for example, it represents the messenger RNA of an expressed pseudogene. In fact, given the co-migration of a crossreacting 47K species on immunoblots of proteins from humans, mice and chickens (and the fact that Alu elements are not present in the last two organisms), it seems unlikely that these Alu elements would be represented in the true 47K product. Furthermore, Yeo *et al.* used antisense RNA expression of the entire cloned cDNA (which includes both elements and a third Alu element downstream of the open reading frame) for functional studies. Given the very large number of Alu insertions within introns, untranslated regions and possibly, in rare cases, coding sequences of human genes^{3,4}, the results of such experiments are difficult to interpret. Of course, should it be the case that translated Alu elements exist in the functional 47K human protein, this would represent the first report of its kind and would have important evolutionary implications⁴.

In our opinion, this example supports the view that

peer review of papers containing DNA or protein sequence data should involve direct evaluation by experts in sequence analysis. This would require direct access of sequence information at the time of review, together with the submitted manuscript, to allow searches to be repeated or performed by a sequence specialist before acceptance for publication.

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YEO *ET AL.* REPLY — We agree that Alu elements are present in the RMSA-1 cDNA sequence that we reported¹. Tugendreich *et al.* suggest that one possible explanation is that we may have cloned an expressed pseudogene. However, we have reviewed our published and unpublished data and firmly believe this not to be the case. We are currently carrying out confirmatory, direct experiments to establish that the RMSA-1 coding sequence contains Alu elements.

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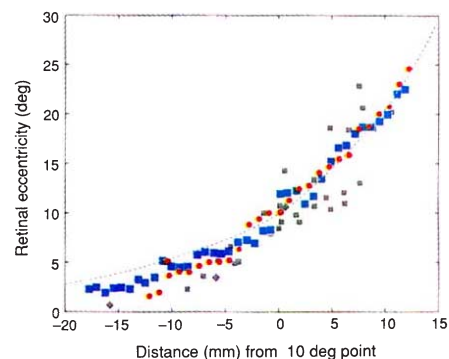
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Erratum



In the Scientific Correspondence by Stephen A. Engel *et al.* of 16 June (“fMRI of human visual cortex”) the labelling of the graph axes were inadvertently omitted from Fig. 2. The correct figure is shown above.

The righter stuff

Arthur C. Clarke

Moon Shot: The Inside Story of America's Race to the Moon. By Alan Shephard and Deke Slayton, with Jay Barbree and Howard Benedict. Introduction by Neil Armstrong. Turner: 1994. Pp. 383. \$21.95.

SOMETIME in the mid-1960s, a young autograph hunter spotted one of the seven Mercury astronauts in the cafeteria of Houston's Manned Spacecraft Center. "Are you Mr X?" he asked hopefully, as he approached, book in hand.

Looking the boy straight in the eye, Mr X answered briefly and firmly: "No".

The astronaut's luncheon guest, who happened to be me, was embarrassed and more than a little shocked. A national hero, uttering such a transparent lie! Now, having read this account of life in the NASA pressure-cooker, I have much more sympathy for my dissembling host. The multiple tensions — technological, political, domestic — created by the programme were almost intolerable; only exceptional men could have withstood them.

Alan Shephard and Deke Slayton were indeed exceptional, even among the astronauts, because they both overcame medical problems that threatened to wreck their careers. Shephard — the first American to leave the atmosphere, even if only for a few minutes — was later grounded because of an obscure inner-ear disease, and for years it seemed that Slayton would never enter space at all, owing to an equally mysterious heart irregularity.

The way they both triumphed over such apparently insuperable difficulties is a moving and inspiring story. Shephard finally reached the Moon on the first flight after the near-disaster of Apollo 13, while Slayton shook hands in orbit with his Russian friends during the Apollo-Soyuz rendezvous, that advance notice of the Cold War's impending demise.

Moon Shot, published to mark the twenty-fifth anniversary of the first manned lunar landing on 20 July 1969, is one of those 'as told to' books which often present problems to the critical reader. How accurate can a re-creation be, if written by someone not actually present at the time? And how many of the conversations given in quotation marks are imagined fictions, even if they are confirmed by the speakers? Few people can remember what they really said decades ago, even — especially

— on some historic occasion.

Despite these caveats, *Moon Shot* is a remarkably convincing work of literary symbiosis, and as exciting as any novel. Concentrating on the personalities in the US — and Soviet — space programmes, it nevertheless gives a clear picture of the technologies involved. Only in the field of nuclear energy has there ever been so



SHADOWLANDS — Buzz Aldrin walking on the Moon. From the cover of *A Man on the Moon* by Andrew Chaikin, a 670-page chronicle of the Apollo missions. Drawing on his interviews with the 23 surviving astronauts, the author, a science journalist, successfully weaves the technological details into a vivid narrative that conveys the human side of the voyages. Michael Joseph/Viking, £17.99, \$24.95.

swift an expansion from small-scale experiments to gigantic national enterprises, as is best demonstrated by the contrasting careers of Robert Goddard and Wernher von Braun.

Just 40 years lay between Goddard's 5-kg liquid-fuelled rocket of 1926 and von Braun's 6,000-ton Saturn V. Not even the most enthusiastic exponents of space travel could have expected so much to happen, so soon. *Nature's* reviewer, "R.v.d. R.W" cannot be harshly criticized

for his comments on the first British book on the subject, P. E. Cleator's 1936 *Rockets Through Space*:

The whole procedure . . . presents difficulties of so fundamental a nature that we are forced to dismiss the notion as essentially unpractical, in spite of the author's insistent appeals to put aside prejudice and to recollect the supposed impossibility of heavier-than-air flight before it was actually accomplished. An analogy such as this may be misleading, and we believe it to be so in this case. . . . We are compelled to join the ranks of those whom Mr Cleator stigmatises as visionless reactionaries [*Nature* 137, 442; 1936].

As a matter of historic interest, the review contains what may have been the first suggestion for one of the most important scientific uses of space technology, the observation of the ultraviolet Universe by rocket-borne instruments. This has indeed revolutionized whole branches of astronomy; all the more surprising, therefore, that — after the International Geophysical Year (1957–58) Earth Satellite programme had been officially announced — Richard van der Riet Woolley made his notorious assertion that space travel was "utter bilge". In the 20 years since writing his review, the Astronomer Royal had, it seems, become even more 'visionless', possibly by becoming too firmly entrenched in the scientific establishment. ("Yes, Professor" could make a fascinating television comedy series, although I fear its appeal would be too limited.)

The US manned space programme also had — and has — its scientific critics, notably Jerome Wiesner, who makes several appearances in *Moon Shot* as a kind of Demon King (other equally deserving candidates escape identification). This is a debate that still continues, as indeed it should. The sorry tale of the Space Shuttle, once supposed to provide cheap, weekly trips into orbit, is a warning of what can happen when engineering and politics collide.

But in the case of Apollo, the combination resulted in a triumph that will be remembered when the nation that achieved it is only a name in the history books. Although it was a triumph rightly earned, by the devoted toil of tens of thousands of anonymous workers as well as the skill and bravery of the few astronauts themselves, a great deal of luck was also involved.

The calculated risk of sending the very first manned Saturn V to the Moon still

seems hair-raising, and Neil Armstrong landed "Eagle" at Tranquillity Base with only 15 seconds of fuel to spare. (He flattered me unduly, when he inscribed his booklet *Wingless on Luna*: "To Arthur, who visualised the nuances of lunar flying long before I experienced them".)

Moon Shot is particularly moving, dealing with the tragic Apollo 1 fire, which, ironically, forced major redesigns without which there would almost certainly have been later disasters. The rescue of Apollo 13, after the mid-flight explosion while it was already heading towards the Moon, still seems a miracle, and the teamwork that made it possible is beyond praise. If the next mission, Apollo 14, had also failed, that would probably have been the end of the programme. It came within a hair's breadth — perhaps literally — of doing so, when the docking mechanisms between the two spacecraft refused to engage.

Yes, NASA and the United States were indeed lucky — they deserved to be. Perhaps it is just as well that the later Apollo missions were cancelled, heart-breaking though it was to all involved, particularly the men who had spent years training for them. Although such speculation is fruitless, the odds against three more successful flights, with such incredibly complex systems, must have been steadily shortening. When we go back to the Moon, it will be with a far more sophisticated technology.

There is a close analogy here with Antarctic exploration. Dog- and man-power took Amundsen and Scott to the South Pole — and, in the latter case, to disaster. When men and women returned there, it was with aircraft and snowcats — and this time, they stayed. So it will be with the Moon; and then we will be ready to go on to the planets.

By a truly incredible — one might almost say eerie — coincidence, the twenty-fifth anniversary of the first Moon landing will coincide with the most dramatic possible demonstration of the long-term (and perhaps short-term) importance of space travel. Even if Jupiter swallows comet Shoemaker-Levy 9 with no more than a few hiccups, the teraton blast should remind us of the fragility, on the cosmic timescale, of our own planet, which has already received two potentially catastrophic impacts this century. (Both in remote parts of Siberia — and how many unknown ones in the oceans?)

We should remember the dinosaurs: unlike them, we have some control over our destiny. Before long, if we set our minds to the task, we will possess the means of deflecting the next, otherwise inevitable hammerblow from space. □

Arthur C. Clarke's *The Snows of Olympus, about the greening of Mars, is to be published by Gollancz in October.*

One cat's view

Harley A. Thronson

The Hubble Wars: Astrophysics Meets Astropolitics in the Two-Billion-Dollar Struggle Over the Hubble Space Telescope. By Eric J. Chaisson. *Harper Collins*: 1994. Pp. 386. \$27.50.

"MANAGING a major space project during a public crisis is like herding cats through the Battle of Antietam," a NASA manager once remarked to me. This is exactly the impression given by this readable and highly personal book, in which Eric Chaisson, an astronomer, details the post-launch crises, infighting, public-relations battles and scientific successes — if not triumph — of history's most expensive

exaggerating the expected performance of Hubble and later on obscuring the serious problems encountered during the commissioning of the crippled observatory. Punctuating the lengthy battle between NASA and the institute are the agency's regular admonitions that the NASA logo be given prominence in any good news (real or imagined) about Hubble. Public education efforts by the agency seem to be non-existent.

On the basis of all this, readers might wonder how the agency ever managed to develop, launch and repair as complex a satellite as Hubble. One should remember however that Chaisson's cat's-eye-view of NASA management is often in opposition to the methods and goals of agency bureaucracy. Chaisson adopts a crusading tone, arguing for greater accuracy in public discussions of Big Science, increased

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Hubble trouble? Technicians work on a replacement solar array for the telescope.

scientific project, the Hubble Space Telescope. The author was one of those cats at Antietam — or, in this case, Baltimore — and one who took extensive notes so that he could re-create his experiences in the maelstrom of the first 18 months or so after the observatory's launch.

Central to Chaisson's version of recent Hubble history, as viewed from his office in the Space Telescope Science Institute, is the antagonism among three different cultures: the bureaucrats, the engineers and the scientists. None escapes his detailed reminiscences.

NASA bureaucracy comes across as particularly unappealing. Upper-level managers appear as inarticulate and confused at best, and vindictive when crossed. Middle-level managers are portrayed as ham-handed apparatchiks, existing primarily to thwart innovation and spawn committees. The agency's public-relations efforts are seen to concentrate first on

public access to scientific enterprises funded by taxpayers' money and a greater role for scientists in the development of these projects. He says little about the political and historical environment within which the agency has had to work and with which the project management has had to contend. Years of dedicated planning are irrelevant to Chaisson's tale, as is a broader discussion of NASA outside his office.

Nevertheless, the memoirs provide an interesting and entertaining companion to Robert W. Smith's *The Space Telescope* (Cambridge University Press, 1989/1993), a weighty history of the telescope before its launch. They contain a colourful description of what it must have been like on the ground during the firestorm surrounding early Hubble operations.

The author is also hard on the engineering community, which, he says, was uncoordinated when faced with the faulty

NASA

satellite. Scientists often act as the heroes, appearing on the scene to rescue their feckless colleagues from yet another technological cul-de-sac. Engineers represent the essence of twentieth-century villainy: faceless, amorphous, obedient in the pursuit of pointless goals and unable to re-examine basic assumptions.

But Chaisson reserves some of his most unflattering characterizations for a few of his scientific colleagues. Exploding tempers, frayed by months of struggle in dealing with the temperamental orbiting robot, are commonplace. Several scientists possess boundless energy only when holding back fascinating information from the public for fear that competitors will pry cosmic secrets from images appearing in *The New York Times*. Most troubling are members of a scientific élite opposed to providing the public with an accurate and accessible description of the Hubble enterprise.

Yet Chaisson is ultimately more sympathetic to his scientific colleagues than to the engineers or administrators — in my view, too much so. Scientists have often had a free ride while working with NASA. Many agency administrators work effectively for years to develop programmes such as Hubble, whereas some scientists consider their own service as limited to appearing on review committees, nodding at appropriate times and writing glowing descriptions of programmes from which they will benefit after engineers and administrators have done all the hard work. Chaisson points out, as others have, that, in the case of Hubble, too few scientists became directly involved in its design, construction and testing. Yet it was the scientific community that was most publicly outraged by Hubble's limitations.

Chaisson follows his own advice by providing accurate and clear technical descriptions of Hubble. The space observatory was by no means a useless piece of expensive orbiting junk before last December's Space Shuttle repair mission, nor is it fully restored, even now. Although the telescope would never accomplish much of what pre-launch hype promised the public and Congress, even with its faulty mirror and other failings, it was a powerful and unique scientific tool. The recent repair mission, which improved its performance greatly, was a much-needed public-relations exercise for Space Station construction techniques. The telescope is not, Chaisson points out, some ultimate astronomical instrument, but is more realistically appreciated as an important step in the continuing exploration of the cosmos.

Also worth noting is the author's skill at weaving several of the early key scientific achievements into what is primarily a political and sociological tale. He is clearly dedicated to a public as knowledgeable about the scientific and technical achieve-

ments as about the human cost and frustration. Nonspecialist readers will get a good, basic description of the astronomical justification for Hubble and of the operation of its instruments. A disappointment though is the quality of the black-and-white illustrations, which are often too small.

There are, then, few admirable characters in this tale of human exploration. (This must have always been the case. One of the attractions of the book is the opening of almost every chapter with an extract from Galileo's writings. The irascible experimental physicist dealt with some of the same political issues almost 400 years ago.) Chaisson's engaging book is a summary of the frustrations and successes, pettiness and generosity, individual enterprise and bureaucratic obfuscation — and eventual technological achievement — of that intensely human activity, the scientific exploration of nature. □

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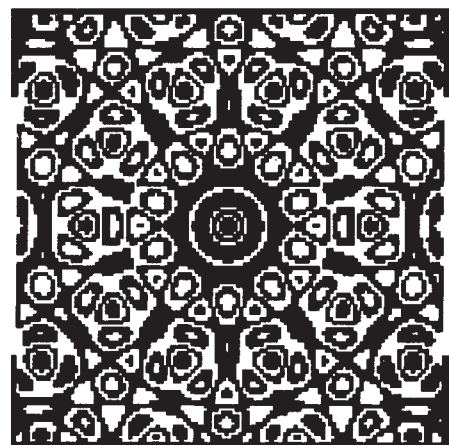
Limitless horizons

Peter Atkins

Designing the Molecular World: Chemistry at the Frontier. By Philip Ball. Princeton University Press: 1994. Pp. 376. \$29.95, £19.95.

CHEMISTRY is a notoriously modest, not to say defensive, subject. Yet it has much to be proud of, both in its intellectual content and in its contribution to modern society. Chemists build forms of matter that perhaps do not exist anywhere else in the Universe, certainly not on Earth. They understand the abilities of matter to undergo transformations, and then subtly deploy these abilities to create novel forms of matter or to generate familiar forms in more economical and environmentally sensitive ways. They provide the foundation for the wealth of nations and materials for advances in medicine and new technologies. Little can be done without matter, and it is the ability of chemists to generate varieties of matter that sustains our progress.

Philip Ball's book illustrates these truths, and does so at a level that anyone who has a willingness to follow will find accessible. He avoids the temptation of bogging down the exposition in the past, and aims directly for the modern. In doing so, he takes us on a tour of the many achievements of chemists over the past couple of decades, and introduces us to the structures of a quite remarkable series



From *Designing the Molecular World*

Fivefold symmetries in the pattern formed by streamlines of fluid flow close to the point at which turbulence develops.

of compounds, and to the understanding of the actions and properties of matter that comes once the structures have been determined. The text moves at a brisk journalist's pace, with a journalist's eye for the important, the eye-catching and the interesting; but the pace does not compromise the science, and the whole book is pervaded by a sense of authority and comprehension.

The book covers a wide selection of topics that are at the forefront of research in modern chemistry. Inevitably there are the fullerenes, with an interesting account of their identification and a sense of their potential. Ball provides just enough background information for non-chemists to see what is involved in the construction and depiction of molecular structures, and the fullerenes are a good example of his style of making a quick but adequate introduction and then moving on to the modern and interesting. He does the same for chemical reactions, and moves the reader swiftly on to an account of catalysts, including the zeolites and their remarkable specificity. The reader will already begin to get a sense of the importance of shape for the determination of function, and to understand that a chemist's seemingly tinker-toy approach to the construction of funny shapes is at root a deeply serious affair, for shape is the doorway to function.

Shape, in its broadest sense, takes on a remarkable form in quasi-periodic solids. Once again, Ball introduces the commonplace by talking a little about conventional solids and the information on their structures that X-ray diffraction provides, but then quickly carries us into the unfamiliar, with a fascinating account of these unusual materials. Nowhere, of course, is intricacy more functional than in living organisms, and Ball shows how chemists can build molecules that make nanotechnology look cumbersome. They can build molecules that can replicate themselves, or act as fingers, tongs or pencil cases or as minute

containers for reactions. Among the more virtuoso syntheses are ring-like molecules that are linked together (including, inevitably, olympene, in which five rings are linked) and large rings that act like railways on which other rings race around, with benzene rings on the track acting like stations. Chemists can emulate ion channels, and, who knows, may one day be able to build a neuron and even a fully organic computer. Already they can emulate biological membranes and, through the construction of special gels and colloids, have built materials that respond to external fields. Thus they can simulate muscles that contract and relax in response to electric fields and make materials that respond similarly to light. Such innovations may prove to be of use in drug delivery, where the casing of a drug may respond to changes in acidity and deliver its load where it is required.

Readers of this book will come away with a clear impression of chemistry's profound and serious contribution to the modern world and of its awesome potential. I recommend it warmly, especially to those on the brink of wondering whether to enter chemistry as a career. They will see the limitlessness of its horizons. □

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What's love got to do with it?

Jeffrey Weeks

Science in the Bedroom: A History of Sex Research. By Vern L. Bullough. BasicBooks: 1994. Pp. 376. \$25.

I FIRST encountered the work of Vern Bullough in what, for lack of a better word, I can only describe as a seminal essay, *Sex in History: A Virgin Field*, first published in 1972. Just as sociologists were then beginning to define a gross absence in social scientific endeavour — the lack of serious study of human sexuality that was more than 'social book-keeping' (who does what to whom and when) — so historians such as Bullough were observing and deploring the silences of the history texts. Only 20 years ago, serious sociological or historical study of sexuality made one, in Ken Plummer's phrase, "morally suspect". That this is less true today is due in large part to the pioneering work of researchers such as Bullough.

Science in the Bedroom surveys what has become a key area for investigation, the role of sex research or sexology itself. The sexological pioneers at the end of the nineteenth and the beginning of the twen-

tieth century saw themselves as engaged in a classically Enlightenment endeavour: to uncover the 'laws of nature' that governed sexual behaviour, to classify its diverse patterns, to establish the rules of normality and abnormality, and thereby to inform public debate. As Bullough notes, pioneers such as Richard von Krafft-Ebing were at least in part encouraged to

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Sex psychologist: Havelock Ellis.

begin their work by the exigencies of forensic medicine. Others such as Ulrichs or Havelock Ellis also had a more political purpose: to provide a more scientific basis to the laws relating to sexuality so that they could be developed in a less moralistic fashion. Bullough rightly pinpoints the importance of the debate on homosexuality to these early sexologists. Homosexuality was a puzzle because it was a cross-cultural and trans-historical phenomenon, acceptable in some forms in some societies and execrated in others, that was startlingly similar to heterosexual forms (object-choice, falling in love, the basis for established relationships and so on); and yet it did not fit easily into the prevailing models of sex as geared to reproduction. Magnus Hirschfeld, the great German sexologist and sex reformer, noted that more than a thousand articles on homosexuality were published between 1895 and 1910 — many of them from his pen. Homosexuality then, as now, provided a litmus test for theories of sexuality, and for the rationality of the legal framework. Hirschfeld's institute for sexual science in Berlin had inscribed above its portals the slogan "Through science to justice", a noble aim unfortunately soon after obliterated by the Nazi onslaught on reason.

More recently, however, this progressive consensus has been subject to attack. Some sexual scientists turned out not to be particularly progressive, especially with regard to homosexuality. More fundamentally, the role of sexology has itself been challenged. The works of Michel Foucault and others have asserted the constitutive role of sexology, not so much describing and anatomizing the various

forms of desire as constructing their importance. Again, homosexuality is a limit case: the new social history has illustrated particularly the way in which the categorization of homosexuality helped to shape the emergence of distinctive homosexual identities from the nineteenth century, with homosexuality seen as the essence of a particular type of person. Sexology, in other words, was complicit with the forms of power and knowledge that imposed definitions as much as they freed human sexual potential. Feminist scholars have similarly sought to show how the apparently liberal views of sexologists actually enforced 'compulsory heterosexuality' on women. Alongside this, a sort of 'grass-roots' sexology has emerged among sexual radicals, emphasizing the importance of self-definition, self-making and self-help often against the nostrums of the would-be scientists of desire. In other words, the hegemony of scientific sex research — even of science itself under the postmodernist onslaught — has been radically challenged.

Bullough's book urbanely scans this fervent sexual-ideological landscape, and will be an extremely useful text for both the general reader and the student. It is a worthy climax to his pioneering work, no longer tilling virgin ground but fertilizing a flourishing landscape. My criticism is that although he provides much of the necessary evidence, his study does not directly tackle the critiques of sexology that I have indicated above. He judiciously measures achievements and failures, but does so within the terms of the sex researchers themselves, treating the scientific endeavour as a noble cause of enlightenment. This certainly accords, as I have indicated, with the self-image of the pioneers, and of their successors, from Freud to Kinsey and beyond. And it would be wrong to deny the achievements of sexology: what we know today about the body, its complicated forms of expression, the role of the mind in shaping sexual expression, and the influence of culture in patterning sexual identities, has been fundamentally shaped by the diligence of sex researchers. But as this list indicates, knowledge of sexual processes does not necessarily tell us how unruly individuals and groups will behave. Sex research in the end is useful only if closely linked to an appreciation of historical and cultural processes. A full history of sex research needs to put these wider processes at its heart. *Science in the Bedroom* has great merits, but there is still a lot of work to be done if we are to understand fully the problematic nature and role of sex research. □

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Folding of nascent polypeptide chains in a high molecular mass assembly with molecular chaperones

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The folding of polypeptides emerging from ribosomes was analysed in a mammalian translation system using firefly luciferase as a model protein. The growing polypeptide interacts with a specific set of molecular chaperones, including Hsp70, the DnaJ homologue Hsp40 and the chaperonin TRiC. The ordered assembly of these components on the nascent chain forms a high molecular mass complex that allows the cotranslational formation of protein domains and the completion of folding once the chain is released from the ribosome.

It is now generally accepted that protein folding and assembly in the cell are mediated by molecular chaperone proteins¹. Two major principles of chaperone action in protein folding have emerged, represented by the members of the heat-shock protein Hsp70 and Hsp60 families^{1,4}.

The Hsp70s recognize short extended peptides enriched in hydrophobic residues^{5,7} and release them on ATP hydrolysis^{8,9}. These chaperones are thought to prevent the premature folding and aggregation of polypeptides during membrane translocation and translation^{10,11}. Although a requirement of Hsp70 for protein folding in the cytosol has never been demonstrated, a number of findings are consistent with such a function: cytosolic Hsc70 (the constitutive Hsp70) is found in association with ribosome-bound polypeptide chains^{12,13} and participates in stabilizing newly synthesized proteins destined for the endoplasmic reticulum (ER) and mitochondria^{14,15}. Hsp70s in the lumen of these organelles bind the incoming polypeptides before their folding^{4,16}. The ability of Hsp70 to prevent protein aggregation *in vitro* has been demonstrated for the *Escherichia coli* homologue DnaK¹⁷. DnaK depends in this capacity on the functional cooperation with the chaperone DnaJ. Homologues of DnaJ are now known to be present in eukaryotic cells as well¹⁸.

The Hsp60s or 'chaperonins'¹⁹ are large oligomeric ring-complexes which mediate the folding of polypeptide chains in an ATP-dependent reaction^{20,21}. They are required for protein folding and assembly in the bacterial cytosol, in chloroplasts and mitochondria^{4,16}. As shown *in vitro*, the Hsp60 of *E. coli*, GroEL, is able to receive an unfolded substrate protein from a complex with DnaK and DnaJ¹⁷. Such a pathway has been demonstrated for the folding of proteins in mitochondria¹⁶. A functional homologue of Hsp60, the large hetero-oligomeric TCP-1 (t-complex polypeptide 1) ring complex (TRiC), has recently been discovered in the eukaryotic cytosol²².

Despite considerable progress in the biochemical and biophysical analysis of molecular chaperones, whether and how these proteins function in the folding of translating polypeptide chains on ribosomes is still unknown. In principle, the amino-terminal portion of the polypeptide could fold spontaneously as it emerges from the ribosome. On the other hand the cooperative nature of the interactions that stabilize the folded structure^{23,24} makes it necessary that a complete folding domain (100–200 amino acids) is available for productive folding. Unfolded or partially

folded polypeptide chains are known to have a strong tendency to undergo off-pathway reactions, such as aggregation^{23,24}. Given the high density of folding protein molecules in the cytosol, this would imply that the growing polypeptide has to be effectively prevented from misfolding and aggregating, until a chain length suitable for productive folding has been synthesized.

We demonstrate that a highly organized chaperone machinery is indeed required for the successful folding of newly translated proteins. Distinct chaperone proteins assemble on the growing polypeptide chain to form a ~1,200K complex that provides the environment for efficient folding.

Cotranslational initiation of folding

Protein synthesis and folding were analysed in a rabbit reticulocyte lysate. Firefly luciferase (62K) was chosen as the model protein because of its rapid and sensitive luminescence assay. Although normally localized in peroxisomes²⁵, luciferase folds to the native state on expression in bacteria and also in the cytosol of yeast²⁶ (J. Höfheld and F.U.H., unpublished results). After initiation with messenger RNA, translation reactions were synchronized by the addition of aurointricarboxylic acid to inhibit re-initiation²⁷ (Fig. 1a). Polypeptide elongation proceeded linearly after a lag phase of about 3 min and short nascent chains became detectable after another 1–2 min (Fig. 1a, top). Translation at 25 °C revealed a delay between the completion of synthesis and the appearance of enzymatically active protein. Fully synthesized luciferase reached the active conformation with a half-time of ~3.5 min (Fig. 1a, top). On translation at 30 °C, this time was reduced to ~2 min (not shown).

We considered the possibility that the rapid folding of the full-length protein may be due to the cotranslational initiation of the folding process. Evidence for this was obtained when the growing polypeptide chains in a synchronized translation were treated with proteinase K. The majority of the incomplete chains of luciferase were degraded, whereas folded, full-length luciferase was resistant (Fig. 1a, bottom and b). However, a distinct protease-stable fragment of ~23K was detected early in translation, when chains up to ~30K had been synthesized. This protease protection reflected the compact folding of an N-terminal domain of luciferase rather than shielding by chaperones, because the 23K fragment behaved as a free monomer on sizing chromatography (not shown). The amount of 23K fragment, generated by proteolysis, decreased over the time course of translation and folding, due to the incorporation of this domain into

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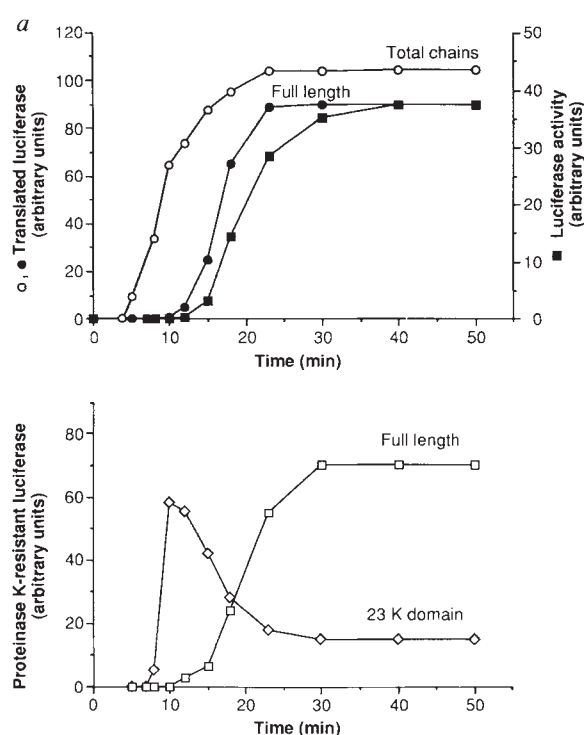


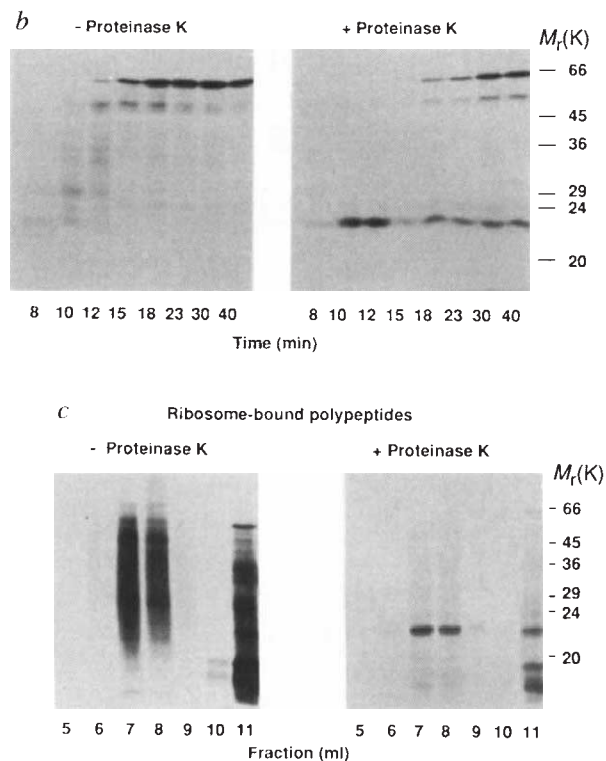
FIG. 1 Cotranslational formation of a compact domain of luciferase. **a**, Synchronized translation of firefly luciferase at 25 °C. Amounts of total luciferase chains, full-length protein and luciferase activity (top), and of protease-resistant luciferase (bottom) are indicated in arbitrary units (averages of four independent experiments). **b**, Polypeptide pattern of a synchronized translation mixture after incubation in the absence or presence of proteinase K. **c**, Formation of the 23K domain of luciferase on isolated ribosome-bound chains.

METHODS. **a**, Firefly luciferase RNA was transcribed from plasmid pGEM-luc (Promega) and translated in 50% nuclease-treated reticulocyte lysate (Promega) in the presence of ^{35}S -methionine (0.8 mCi ml^{-1}) as recommended by the manufacturer. The reaction volume was adjusted with buffer A (20 mM HEPES-KOH pH 7.4, 100 mM KAcO and 1 mM $\text{Mg}(\text{AcO})_2$). Where indicated, translations were synchronized by addition of 75 μM aurintricarboxylic acid (ATCA) 3 min after initiation of translation by the addition of luciferase mRNA. The rates of translation were ~ 60 and 95 residues per min at 25 °C and 30 °C, respectively. Unless otherwise indicated, translation was stopped at the indicated times by threefold dilution of aliquots into ice-cold buffer B (25 mM Tris-phosphate pH 7.8, 2 mM *trans*-1,2-cyclo-hexanediaminetetraacetate (CDTA) and 1 mg ml^{-1} BSA) containing 2 mM methionine and either 2 mM cycloheximide or 2 mM puromycin. Luciferase activity was determined at 25 °C^{31,35}. Amounts of total translation products and of full-

length luciferase were determined by Phosphorimager quantification. The first 270 residues of luciferase contain 11 out of the 14 methionines of the protein. Specific enzyme activities (SEA) were calculated from the amounts of full-length protein based on the specific radioactivity of ^{35}S -methionine in the reaction mixture. The SEA of translated luciferase was equivalent to that of a purified protein standard. **b**, Aliquots of a synchronized translation mixture were stopped as in **a** and half of each reaction was treated with proteinase K ($10\text{ }\mu\text{g ml}^{-1}$) at 4 °C^{35,47}, followed by 15% SDS-PAGE and fluorography. **c**, A 50 μl unsynchronized translation mixture (20 min at 25 °C) was diluted threefold in buffer C (buffer A plus 5 mM $\text{Mg}(\text{AcO})_2$, 0.5 mM cycloheximide, 1 mM DTT, 1 U μl^{-1} RNasin, 1 mM PMSF, 2 $\mu\text{g ml}^{-1}$ aprotinin, 0.5 $\mu\text{g ml}^{-1}$ leupeptin) and ATP was removed by incubation with apyrase ($0.5\text{ U }\mu\text{l}^{-1}$ for 5 min at 25 °C). The reaction mixture was then loaded onto an 11 ml 20–45% sucrose gradient in buffer D (buffer A plus 5 mM MgCl_2 , 1 mM EGTA) and centrifuged for 3 h 45 min in a SW-41 rotor (40,000 r.p.m., 4 °C). Absorbance of the 1 ml fractions at 254 nm indicated a monoribosomal peak eluting at 7–8 ml. Monoribosomes were produced because mRNA was in excess. Aliquots of the fractions were incubated for 10 min at 4 °C with or without proteinase K as in **b**. Nearly full-length nascent chains appear larger than free full-length luciferase because of the attached tRNA.

Post-translational completion of folding

Because the carboxy-terminal 20–40 residues of the nascent polypeptide are sequestered by the ribosome²⁸, it seemed likely that the release of the polypeptide from the ribosome is a prerequisite for complete folding. To test this, we used the fact that translation products of mRNAs lacking a stop codon remain ribosome-bound as peptidyl-transfer RNAs^{29,30}. On translation of a truncated mRNA encoding a luciferase protein lacking the 11



length luciferase were determined by Phosphorimager quantification. The first 270 residues of luciferase contain 11 out of the 14 methionines of the protein. Specific enzyme activities (SEA) were calculated from the amounts of full-length protein based on the specific radioactivity of ^{35}S -methionine in the reaction mixture. The SEA of translated luciferase was equivalent to that of a purified protein standard. **b**, Aliquots of a synchronized translation mixture were stopped as in **a** and half of each reaction was treated with proteinase K ($10\text{ }\mu\text{g ml}^{-1}$) at 4 °C^{35,47}, followed by 15% SDS-PAGE and fluorography. **c**, A 50 μl unsynchronized translation mixture (20 min at 25 °C) was diluted threefold in buffer C (buffer A plus 5 mM $\text{Mg}(\text{AcO})_2$, 0.5 mM cycloheximide, 1 mM DTT, 1 U μl^{-1} RNasin, 1 mM PMSF, 2 $\mu\text{g ml}^{-1}$ aprotinin, 0.5 $\mu\text{g ml}^{-1}$ leupeptin) and ATP was removed by incubation with apyrase ($0.5\text{ U }\mu\text{l}^{-1}$ for 5 min at 25 °C). The reaction mixture was then loaded onto an 11 ml 20–45% sucrose gradient in buffer D (buffer A plus 5 mM MgCl_2 , 1 mM EGTA) and centrifuged for 3 h 45 min in a SW-41 rotor (40,000 r.p.m., 4 °C). Absorbance of the 1 ml fractions at 254 nm indicated a monoribosomal peak eluting at 7–8 ml. Monoribosomes were produced because mRNA was in excess. Aliquots of the fractions were incubated for 10 min at 4 °C with or without proteinase K as in **b**. Nearly full-length nascent chains appear larger than free full-length luciferase because of the attached tRNA.

C-terminal amino acids (FL- Δ C11), $\sim 50\%$ of the polypeptide chains sedimented with the ribosomes through a dense sucrose solution. The ribosome-bound FL- Δ C11 was indeed enzymatically inactive (Fig. 2a), whereas the FL- Δ C11 protein in the supernatant fraction had the same enzymatic activity as full-length luciferase (not shown). The isolated ribosome-FL- Δ C11 complex was used to determine whether the completion of folding on polypeptide release requires ATP, suggesting the involvement of molecular chaperones (Fig. 2a). Very little luciferase activity was detected after a 15 min incubation of the ribosome-peptidyl-tRNA complex at 30 °C, both in the presence and absence of Mg-ATP. Addition of puromycin induced the release of $\sim 95\%$ of the nascent chains within 5 min (not shown), but this resulted only in a small stimulation of luciferase activity. However, the addition of Mg-ATP together with puromycin caused the efficient production of active luciferase (Fig. 2a). Because ATP does not enhance the inefficient spontaneous refolding of chemically denatured luciferase³¹, these results sug-

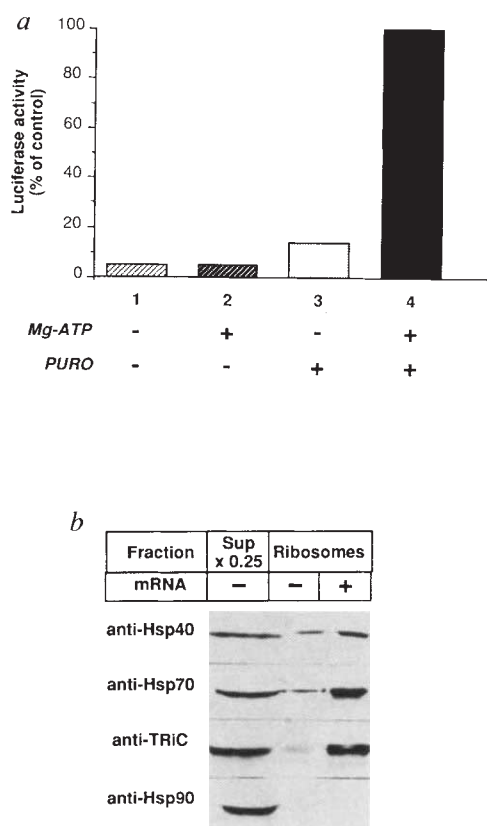


FIG. 2 *a*, Requirement of chain release from the ribosome and Mg-ATP for completion of folding. Isolated ribosomes bearing C-terminally truncated firefly luciferase FL- Δ C11 were treated at 30 °C with either 2 mM cycloheximide or 2 mM puromycin in the presence or absence of 5 mM $MgCl_2$, 1 mM ATP (Mg-ATP), as indicated. Enzyme activities were measured 15 min after initiating folding and are expressed as per cent of the activity in the reaction mixture treated with puromycin and Mg-ATP. On the basis of the specific enzyme activity of FL- Δ C11, about 70% of the chains in the ribosomal pellet folded to the native state under these conditions. *b*, Association of molecular chaperones with translating ribosomes. Ribosomes were isolated from unsynchronized translation reactions incubated in the presence (+mRNA) or absence (-mRNA) of luciferase mRNA. The amounts of Hsp40, Hsp70, TRiC and Hsp90 present in the ribosomal fractions and in the post-ribosomal supernatant of untreated reticulocyte lysate (Sup) were determined. The amount of ribosomes analysed is equivalent to 4 times the amount of supernatant fraction shown.

METHODS. *a*, Luciferase protein lacking the 11 C-terminal amino-acid residues (FL- Δ C11) was synthesized at 25 °C from pGEM-luc RNA linearized with *Eco*NI. The reaction was stopped with cycloheximide and ribosome-bound FL- Δ C11 was isolated from ATP-depleted reticulocyte lysate (see Fig. 1; very similar results were obtained when ATP-depletion was omitted). Lysate was diluted threefold with buffer C, 0.5 M KCl, centrifuged first for 10 min at 16,000 g to remove aggregates and then for 20 min at 120,000 r.p.m. (TL-120.1, 4 °C) through 350 μ l of 25% sucrose, buffer C, 0.5 M KCl. Ribosomal pellets were washed twice in buffer C, 0.5 M KCl and resuspended in the initial volume of buffer C. About 50% of the FL- Δ C11 was recovered in the ribosomal fraction. Puromycin treatment released 95% of nascent chains after 5 min incubation at 30 °C (not shown). *b*, Translation was done for 20 min at 25 °C, and ribosomes were isolated as above. The amount of chaperones was determined by western blot analysis using polyclonal rabbit antisera against Hsp40³² and Hsc70, chicken antibodies against TRiC and a mouse monoclonal against Hsp90 (Stressgen). Results obtained with the enhanced chemiluminescence (ECL) system (Amersham) were quantified by laser densitometry using purified proteins as standards^{35,48,49}. The anti-Hsc70 antibody recognizes both the constitutive and the inducible form of Hsp70. The approximate concentrations of chaperones in the total lysate are: Hsc70/Hsp70, 2 μ M; TRiC, 0.7 μ M; and Hsp90, 2 μ M. Hsp40 could not be quantified because of the lack of purified standard protein.

gest that chaperone proteins remain associated with the isolated, ribosome-bound luciferase and mediate the completion of folding in an ATP-dependent manner once the full-length polypeptide is available.

Chaperone assembly on the nascent polypeptide

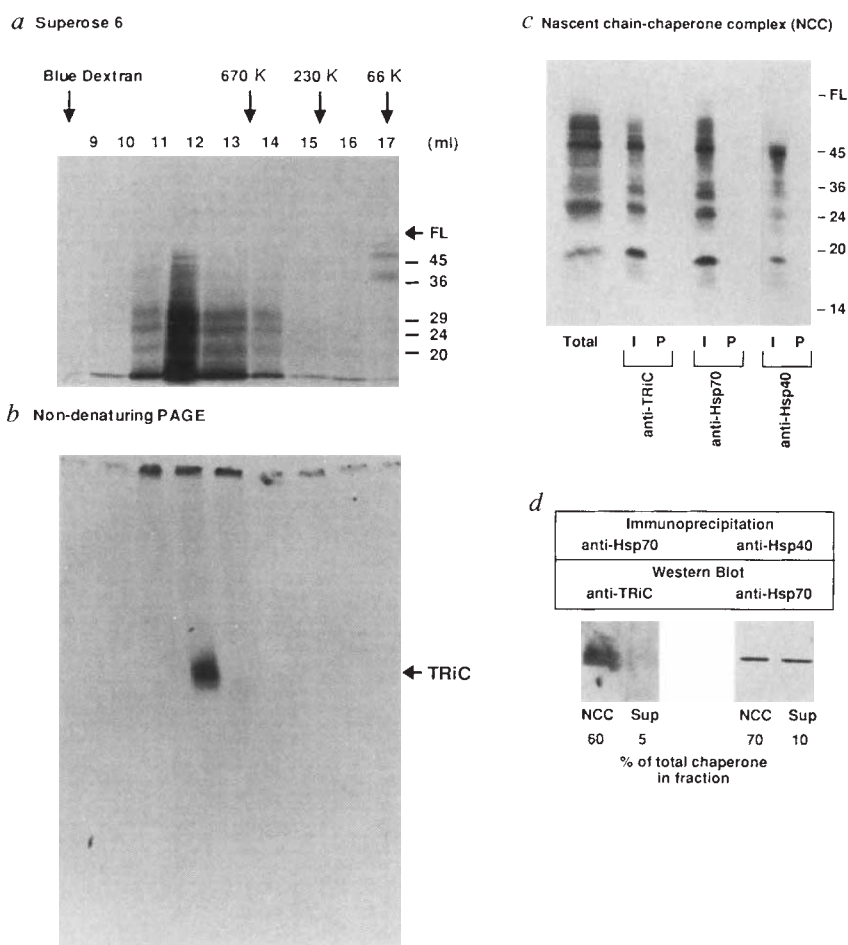
In an approach to identify the cellular machinery involved in the folding of nascent luciferase, ribosomes were isolated from reticulocyte lysates that had been incubated under translation conditions in the presence or absence of luciferase mRNA. The association with the ribosomes of the major chaperone proteins Hsp90, Hsc70/Hsp70 (hence Hsp70) and the cytosolic chaperonin TRiC was analysed by immunoblotting. Hsp40, a recently described mammalian homologue of *E. coli* DnaJ^{32,33}, was included in the analysis. These chaperones are present in the lysate at concentrations of ~0.7–2 μ M (see legend to Fig. 2), whereas the concentration of ribosomes is about 0.2 μ M³⁴. About 10–15% of the total Hsp70 and Hsp40 as well as 20–25% of TRiC were specifically associated with the translating ribosomes, whereas Hsp90 was not detected in the ribosome fraction (Fig. 2b). These data suggest that a set of chaperone components functionally and structurally related to bacterial DnaK, DnaJ and GroEL participate in the folding of nascent polypeptides in the eukaryotic cytosol.

To examine whether some or all of these chaperone proteins interacted directly with the nascent luciferase, ribosome-peptidyl-tRNA complexes were isolated by sucrose gradient centrifugation from an unsynchronized translation mixture stopped with cycloheximide. The relative accumulation of defined species of nascent chains is apparently due to translational pausing (also see Fig. 1b). The nascent polypeptides were released from the ribosomes by treatment with puromycin and analysed by gel filtration chromatography (Fig. 3a). About 65% of the luciferase chains loaded were recovered from the column, ranging in size from 12 to 60K. Surprisingly, they were found in a defined high molecular mass complex of ~1,200K (12 ml fraction). On native polyacrylamide gel electrophoresis (PAGE) this complex migrated as a uniform species with a mobility very similar to that of the ~950K chaperonin TRiC³⁵ (Fig. 3b). The nascent polypeptides in the 1,200K fraction could indeed be coimmunoprecipitated with 20–30% efficiency by a polyclonal anti-TRiC antibody (Fig. 3c; see legend for quantification).

Notably the same spectrum of chains that were precipitated by anti-TRiC were also precipitated by the anti-Hsp70 antibody (Fig. 3c). A similar polypeptide pattern was precipitated with antibody against Hsp40, albeit with somewhat lower efficiency (Fig. 3c). Because unfolded proteins bound to eukaryotic Hsp70 or bacterial DnaK and DnaJ fractionate at a smaller size of ~230K¹⁷ (see Fig. 5), these findings strongly suggest that Hsp70, Hsp40 and TRiC are all present in a specific high molecular mass assembly with nascent polypeptides. Notably, proteinase K treatment of the luciferase chains in this complex revealed the presence of the folded 23K domain (not shown), consistent with the known accessibility of chaperone-bound polypeptides to this protease^{17,21,35}.

The presence of Hsp70 and TRiC in a unique complex with the same nascent chains could be confirmed by the coimmunoprecipitation of 60% of the TRiC in the ribosome/nascent chain fraction with anti-Hsp70 antibody (Fig. 3d). Notably, the association between the two chaperones during coimmunoprecipitation was twice as stable as the interaction of either protein with the nascent chains (see above). This may suggest a direct interaction between Hsp70 and TRiC in the context with the nascent polypeptide. In contrast, the Hsp70 and TRiC remaining in the postribosomal supernatant of the reticulocyte lysate showed only a weak interaction. Similarly, the antibody against Hsp40 coimmunoprecipitated 70% of the Hsp70 from the complex with nascent chains (Fig. 3d), but only ~10% of the Hsp70 in the postribosomal supernatant.

FIG. 3 Formation of a high molecular mass nascent chain/chaperone complex. ^{35}S -labelled polypeptide chains bound to isolated ribosomes were released with 2 mM puromycin and subjected to sizing chromatography. The fractionation of molecular mass standards is indicated. Aliquots of 30 μl and 50 μl were analysed by 12% SDS-PAGE (a) and non-denaturing-PAGE (b), respectively. The arrow in *b* indicates the migration of purified bovine TRiC 35 . *c*, Coimmunoprecipitation of the polypeptides in the nascent chain/chaperone complex (NCC) with anti-TRiC, Hsp70 and Hsp40 antisera. The efficiency of precipitation with these antisera was 30%, 35% and 21% of the total nascent chains, respectively, as judged by Phosphorimager quantification. I, Immune serum; P, preimmune serum; FL, full-length firefly luciferase. *d*, Interaction between different chaperones in the NCC and in the post-ribosomal supernatant revealed by coimmunoprecipitation. The amounts of TRiC and Hsp70 precipitated with antisera against Hsp70 and Hsp40, respectively, were determined by western blotting and are expressed in per cent of the total TRiC and Hsp70 present in the NCC and post-ribosomal supernatant fractions. **METHODS.** Ribosome-bound chains were isolated from 100 μl of an unsynchronized translation reaction mixture (15 min, 25 °C) by sucrose gradient centrifugation (Fig. 1). *a*, Nascent chains were released with puromycin and separated on a Superose 6 column (Pharmacia) equilibrated in buffer A, 10% glycerol. A volume of 0.25 ml was loaded and 1 ml fractions were collected. *b*, Non-denaturing gel electrophoresis (3–15% polyacrylamide) was as described 47 . *c*, Samples (90 μl) of column fraction 12 were coimmunoprecipitated with chaperone antisera, followed by analysis on 15% SDS-PAGE together with 30 μl of fraction 12 (total). The presence of immunoglobulin heavy chains is responsible for the compression in the 50–55K range where anti-Hsp40 is used. For immuno-precipitation, samples were diluted to 500 μl with TBS (25 mM Tris-HCl pH 7.4, 137 mM NaCl and 2.7 mM KCl) and incubated with 5 μl of immune or preimmune serum for 1 h at 4 °C. The samples were then centrifuged at 16,000 g for 10 min and incubated with 5 μl (packed volume) of protein A-Sepharose for 1 h at 4 °C. Anti-Hsp70, anti-TRiC and preimmune rabbit antibodies were covalently coupled to protein A-Sepharose beads 50 . Sepharose beads were washed 5 times with 1 ml of TBS, 0.05% Triton X-100 and once with TBS; bound proteins were eluted using non-reducing SDS sample buffer and treated with RNaseA (1 mg ml $^{-1}$) for 10 min at 25 °C before



SDS-PAGE. *d*, The NCC was immunoprecipitated with antibodies against Hsp70 or Hsp40 bound to protein A-Sepharose. An equivalent amount of post-ribosomal supernatant (Sup) was immunoprecipitated in parallel. About 70–80% of the total Hsp70 and Hsp40 was precipitated. Immunoprecipitates were subjected to western blotting using anti-bovine TRiC antibodies raised in chicken or a mouse monoclonal anti-Hsp70 antibody (Stressgen), as indicated. Amounts of TRiC and Hsp70 were quantified using the purified proteins as standards.

Requirement of chaperones for folding

To test whether the Hsp70/Hsp40 and TRiC chaperone systems were indeed required for productive folding, these proteins were removed from reticulocyte lysates by immunodepletion. Treatment of lysates with antibodies against Hsp70, Hsp40 or TRiC bound to protein A-Sepharose resulted in ~70% depletion of these proteins without causing a significant decrease in the concentrations of the remaining two chaperones (not shown). Although the Hsp70 depletion did not cause an inhibition of translation, a 70% reduction in the final specific activity of the translated full-length luciferase was measured (Fig. 4a). Only little of the non-native luciferase protein was found in sedimentable aggregates, however (not shown). Other components in the lysate may still be able to maintain the solubility of the newly synthesized protein, although these luciferase chains are no longer competent to fold. Addition of purified Hsp70 to the depleted lysate before initiation of translation reconstituted the efficient folding of the enzyme. In contrast, Hsp70 was without effect when added after completion of translation. Likewise, the cotranslational addition of purified TRiC did not compensate for the loss of Hsp70 function. These results demonstrate that

Hsp70 is required during translation to allow the correct folding of the newly synthesized protein. Interestingly, an equivalent defect in folding was caused by the immunodepletion of Hsp40 (not shown; see Fig. 5b).

The removal of TRiC also caused a decrease in the final specific activity of luciferase by 65% without affecting the efficiency of translation (Fig. 4b). The addition of purified Hsp70 to the TRiC-depleted lysate did not compensate for the loss of TRiC function. Readdition of purified TRiC at the beginning of translation fully restored the normal yield of the folding reaction, whereas addition 20 min after initiation of translation resulted only in a 30% restoration. Thus, unlike Hsp70, the chaperonin has a limited ability to act after completion of polypeptide synthesis. Notably, the removal of TRiC did not abolish the formation of the protease-resistant 23K domain of luciferase (not shown). However, the cotranslational folding of this domain in the undepleted lysate is likely to occur in association with TRiC, because nascent chains shorter than 23K were already in a complex with the chaperonin (see Fig. 3). Apparently, both Hsp70 (together with Hsp40) and TRiC need to interact with the nascent polypeptide for efficient folding. A post-translational func-

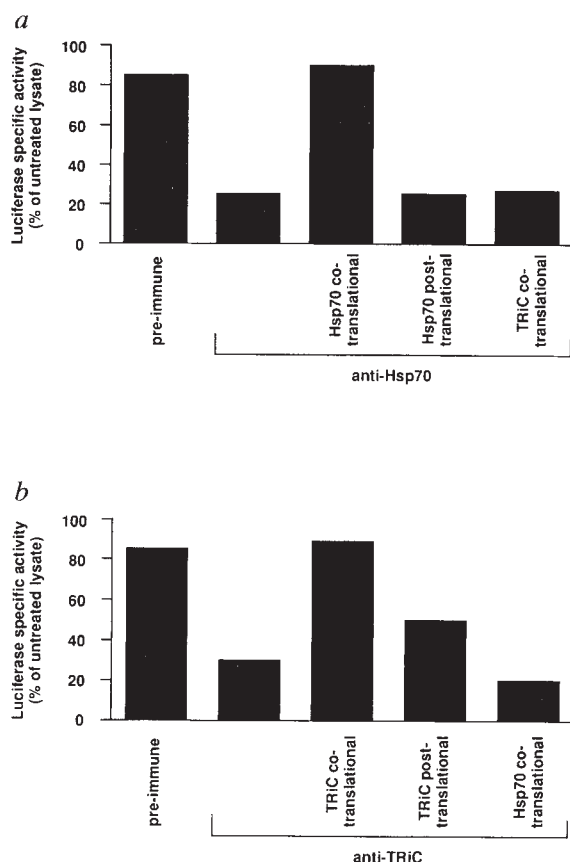


FIG. 4 Chaperone proteins are required for luciferase folding. Reticulocyte lysate was immunodepleted of either Hsp70 (a) or TRiC (b). Purified bovine Hsc70 or TRiC (3 μ M and 1 μ M final concentrations, respectively) were added to aliquots of the depleted lysate before synchronized translation (cotranslational addition) or 20 min after initiation of translation (post-translational addition). Specific enzyme activities are expressed as per cent of the specific activity in control translations performed with untreated lysate.

METHODS. Reticulocyte lysates were incubated with protein A-Sepharose crosslinked to IgG from either preimmune or anti-Hsp70 immune sera (a) or preimmune or anti-TRiC sera (b). The antibodies coupled to protein A-Sepharose beads (40 μ l packed volume) were washed 3 times with buffer A and incubated for 2 h at 4 °C with 40 μ l lysate in a compact reaction spin column (USB). The lysate was recovered by spinning the column dry. Depletion of the endogenous chaperones was assessed by western blot analysis of the lysates using the mouse monoclonal against Hsc70/Hsp70 or a rat monoclonal antibody against TCP-1 (a gift of K. Willison). Results generated by the ECL detection system were quantified by densitometry. The extent of Hsc70/Hsp70 depletion in a was 70–80% and of TRiC depletion in b was 60–70%. The lysate contents of TRiC in a and of Hsp70 in b were unaffected. The depletion of Hsp40 (~70%) was done as described for Hsp70 and TRiC using a polyclonal Hsp40³² antiserum and preimmune serum as control (not shown). Synchronized translation was at 25 °C for 35 min. Translation was complete after 30 min. The amounts of full-length luciferase were quantified and luciferase activities were determined. Specific enzyme activities were calculated as enzyme activity : amount of full-length protein.

tion of TRiC may be more significant in the folding of proteins considerably smaller than luciferase.

Defined order of chaperone interactions

Did the interaction of Hsp70/Hsp40 and TRiC with the growing polypeptide occur in an ordered sequence? To test whether the chaperonin is able to associate with a very short ribosome-bound chain, a truncated form of luciferase was produced comprising only the N-terminal 77 amino acids of the protein (FL-77). Nascent FL-77 is expected to expose ~40 residues outside the ribo-

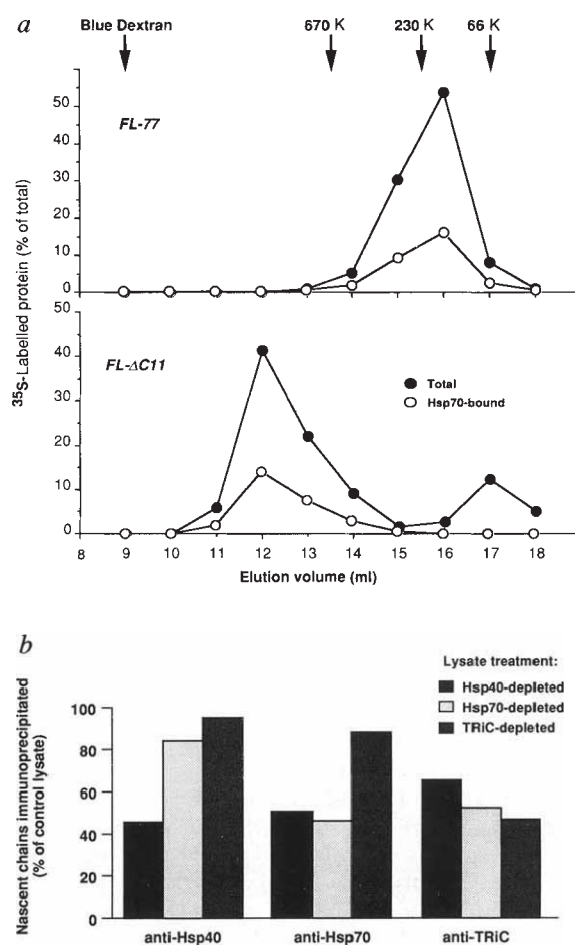
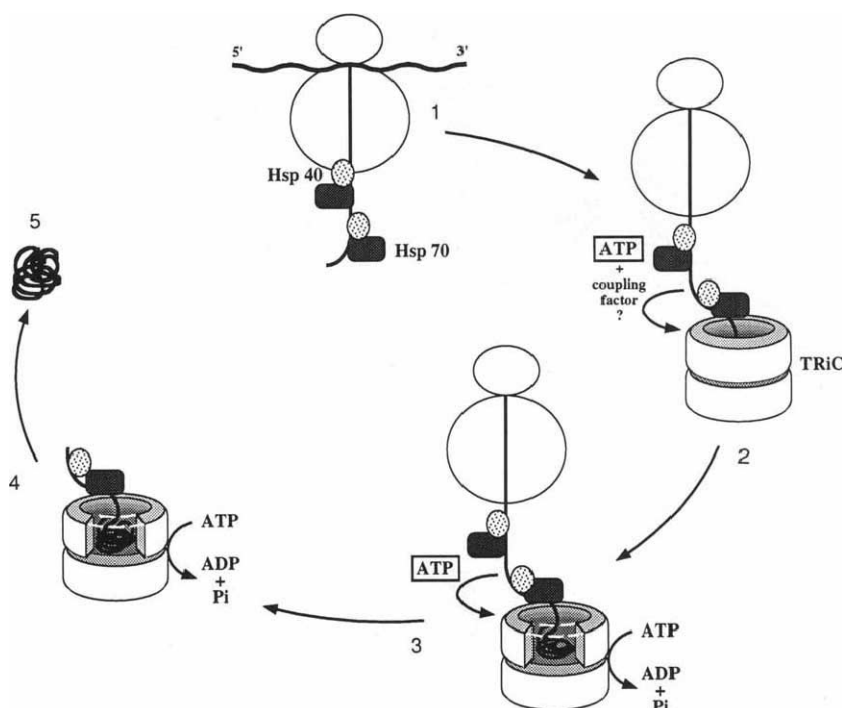


FIG. 5 Sequential interaction of the elongating polypeptide with Hsp70 and TRiC. **a**, Dependence of the association of chaperones on the chain length of the ribosome-bound polypeptide. Gel filtration profiles of total nascent FL-77 and FL- Δ C11 chains and the amounts of these proteins coimmunoprecipitated with anti-Hsp70 are shown. **b**, Requirement of Hsp70 and Hsp40 for binding of nascent chains to TRiC. Reticulocyte lysates were immunodepleted of Hsp40, Hsp70 or TRiC as described in Fig. 4. Control lysates were treated with preimmune serum. The amounts of chaperone-bound polypeptides in the Hsp40, Hsp70 and TRiC-depleted lysates are expressed as a percentage of the amounts coimmunoprecipitated from control lysates treated with preimmune serum.

METHODS. **a**, pGEM-luc was linearized with *Eco*NI (FL- Δ C11; Fig. 2) or with *Hin*II, generating a polypeptide comprising amino acids 1–77 of luciferase (FL-77). After translation, ribosomes carrying the 77-mer chain (8.4K) or the FL- Δ C11 (61K) protein were isolated as in Fig. 3. Puromycin-released chains were analysed on Superose 6 (see Fig. 3) and co-immunoprecipitated with Hsp70 antibody as indicated. The interaction of FL- Δ C11 with TRiC was ascertained by native PAGE. The distribution of ³⁵S-labelled protein in the column fractions was analysed by SDS-PAGE and Phosphorimager analysis. Amounts are given in per cent of total luciferase protein present in the column fractions. The recovery of luciferase polypeptides from the sizing column was ~70%. **b**, Unsynchronized translations (30 μ l) were done for 25 min in either immune- or preimmune-treated lysates and stopped by twofold dilution into buffer A containing 2 mM puromycin and 10 U apyrase. One-fifth of the reaction mixture was used to quantify the total amount of nascent chains, which was similar under the different conditions tested. Nascent chains of ~15–60K were present (see Fig. 3). The remainder of the reaction mixtures was divided into aliquots containing equal amounts of chains and their interaction with molecular chaperones was analysed by coimmunoprecipitation. Immunoprecipitates as well as total polypeptide chains were analysed by SDS-PAGE and Phosphorimager quantification. The extent of immunodepletion of Hsp40 and Hsp70 was 70% and of TRiC 65%. The efficiency of coimmunoprecipitation of the total chains in the preimmune-treated lysate was 20%, 30% and 27% for Hsp40, Hsp70 and TRiC, respectively (see also Fig. 3c).

FIG. 6 A model for the folding of luciferase translated in the eukaryotic cytosol. (1) The polypeptide chain emerging from the ribosome binds first to Hsp40 and Hsp70. (2) An N-terminal segment of the polypeptide chain interacts with TRiC on ATP-dependent release from Hsp70/Hsp40. A cytosolic factor(s) may be involved in functionally coupling both chaperone systems. (3) As translation proceeds, ATP-dependent folding of an N-terminal domain of luciferase occurs, presumably within the central cavity of the TRiC cylinder, while C-terminal regions of the polypeptide chain remain associated with Hsp40/Hsp70 in an unfolded conformation. With luciferase as substrate, TRiC has also a limited capacity to act post-translationally (not shown). (4) On completion of translation, the newly made polypeptide dissociates from the ribosome and is available for completion of folding in association with TRiC. (5) This results in the formation of the free native protein.



some. On release from isolated ribosomes, FL-77 was found exclusively in a roughly 200K complex (Fig. 5a), from which it could be coimmunoprecipitated with anti-Hsp70 antibody. In contrast, the analysis of nascent FL-ΔC11 confirmed that the nearly full-length protein was in the ~1,200K complex containing Hsp70 and TRiC. These observations provide insight into the structural requirements of the two chaperone systems for their interaction with translating luciferase: Hsp70, in cooperation with Hsp40, is able to bind to very short nascent chains, whereas TRiC appears to be unable to assemble onto the nascent chain until a chain-length of about 100–150 residues has been reached.

It seemed possible that Hsp70/Hsp40 and TRiC associate with the ribosome-bound chain independently of one another, determined mainly by the length of the polypeptide substrate. To test this, we investigated whether the removal of one chaperone protein affected the association of nascent luciferase with the remaining two components. Strikingly, the partial depletion of Hsp70 from the lysate resulted in a corresponding reduction in the binding of nascent chains to TRiC in an unsynchronized translation mixture (Fig. 5b), equally affecting the complete spectrum of chain lengths of ~15–60K (not shown). In contrast, the association of nascent chains with Hsp40 was undiminished. Removal of TRiC was without effect on the binding of nascent luciferase to Hsp70 and Hsp40 (Fig. 5b). As expected, on size-exclusion chromatography nascent chains were then found in a complex of ~230K (not shown). Depletion of Hsp40, by contrast, decreased the interaction of nascent chains both with Hsp70 and with TRiC (Fig. 5b), suggesting that Hsp40 is involved in the loading of Hsp70 onto the polypeptide emerging from the ribosome.

Together these results demonstrate that Hsp70/Hsp40 interact first with the elongating polypeptide thus mediating the complex formation with TRiC and subsequent folding. This transfer step may be helped by a direct structural and functional coupling between Hsp70/Hsp40 and TRiC, directing the nascent chain through an organized pathway of chaperone-assisted protein folding.

Discussion

The machinery mediating the folding of nascent polypeptides in the eukaryotic cytosol appears to combine two distinct principles

of chaperone action in a structurally and functionally coupled assembly. The components involved in this pathway have been highly conserved in evolution. Hsp70, the DnaJ-homologue Hsp40 and the double-ring chaperonin TRiC form a high molecular mass complex with the ribosome-bound polypeptide that mediates protein folding in an ATP-dependent process (Fig. 6). Hsp40 and Hsp70 bind first to the nascent chain and subsequently mediate the interaction of the growing polypeptide with the TRiC cylinder. This may reflect a basic principle of cellular protein folding: the ordered cooperation of different classes of chaperone proteins on the growing substrate protein. Additional factors may associate with this chaperone machinery to catalyse the covalent and non-covalent modification of newly synthesized proteins.

Our results provide insight into the constraints imposed on protein folding by translation. A discrete domain of luciferase is able to fold cotranslationally once about 250–300 residues of the polypeptide chain have been synthesized. The remaining part of the 550-residue protein acquires its stable tertiary structure only after the release of the fully synthesized chain from the ribosome. Presumably, the first important function of chaperone binding is to stabilize the segments of the elongating polypeptide, not yet able to fold, until a chain-length suitable for productive folding has been reached. Our data would assign this function to the Hsp70/Hsp40 system, consistent with the ability of Hsp70 to recognize extended peptides^{5,7} and with its reported association with nascent polypeptide chains^{12,13}. Removal of Hsp70 or Hsp40 renders the newly synthesized luciferase incapable of folding, apparently by interrupting its ordered transfer to TRiC. When bound to the substrate protein, the Hsp70 interacts efficiently with Hsp40. This DnaJ homologue seems to function in mediating the binding of Hsp70 to polypeptide substrate. It thus appears likely that Hsp70 functionally cooperates with Hsp40, and perhaps other DnaJ homologues, in maintaining the translating chain (or parts thereof) unfolded and competent for folding, as proposed for DnaK and DnaJ in the *E. coli* cytosol^{17,36}.

Hsp70/Hsp40 are necessary but not sufficient for productive folding. In order to reach the native state, the growing polypeptide has to interact with the chaperonin complex TRiC. Like the bacterial GroEL^{37,38}, TRiC possesses a large central cavity which has recently been demonstrated to accommodate the unfolded

substrate protein³⁹. Stable association with the chaperonin is thought to require a critical length of polypeptide, able to adopt a compact structure⁴. Folding would occur on ATP-dependent release of the bound protein into the cavity. We propose that Hsp70/Hsp40 transfer the growing polypeptide chain into this protected space. This transfer step appears to occur before the cotranslational folding of a domain of luciferase is completed. A eukaryotic homologue of the bacterial nucleotide exchange protein GrpE, acting on Hsp70^{17,40}, may be involved in this reaction as a coupling factor (Fig. 6). In contrast to GroEL, the eukaryotic chaperonin is a hetero-oligomer of several structur-

ally related subunits^{35,41–43}. This complexity may be related to the potential of TRiC to interact with other chaperone proteins in a highly regulated folding pathway. TRiC has been implicated in the folding of abundant cytosolic proteins such as actin and tubulin^{44–46}, but, as shown here, its function may not be restricted to these proteins.

Our results indicate that soluble proteins in the eukaryotic cytosol are guided to their final conformation through a highly organized chaperone pathway. It is quite possible, however, that distinct parallel pathways of protein folding exist, involving different sets of cooperating components. □

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LETTERS TO NATURE

Compact OH megamaser and probable quasar activity in the galaxy Arp 220

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Arp 220 is the prototype far-infrared ultraluminous galaxy, and the origin of its luminosity—a burst of massive star formation or a quasar obscured by a layer of dense gas and dust—has been the subject of much debate^{1,2}. It also contains the prototypical OH megamaser³—an extremely luminous version of masers (microwave lasers) commonly found in our own galaxy. It has been thought that the dense gas in the inner few hundred parsecs of megamaser galaxies acts as a low-gain masing screen, pumped by the far-infrared radiation, which amplifies background continuum emission from the nuclear regions^{4–6}. Here we show, using new

very-long-baseline interferometry observations, that the OH line peak in Arp 220 originates in a structure ≤ 1 pc across, positionally aligned with a weak continuum feature, and that most of the emission originates on scales of ≤ 10 pc. These results imply that the maser is physically 10–100 times smaller than previously thought^{5,6}, strongly suggesting that much of the far-infrared radiation from Arp 220 arises in a very small region, possibly a dense molecular torus, surrounding a quasar nucleus.

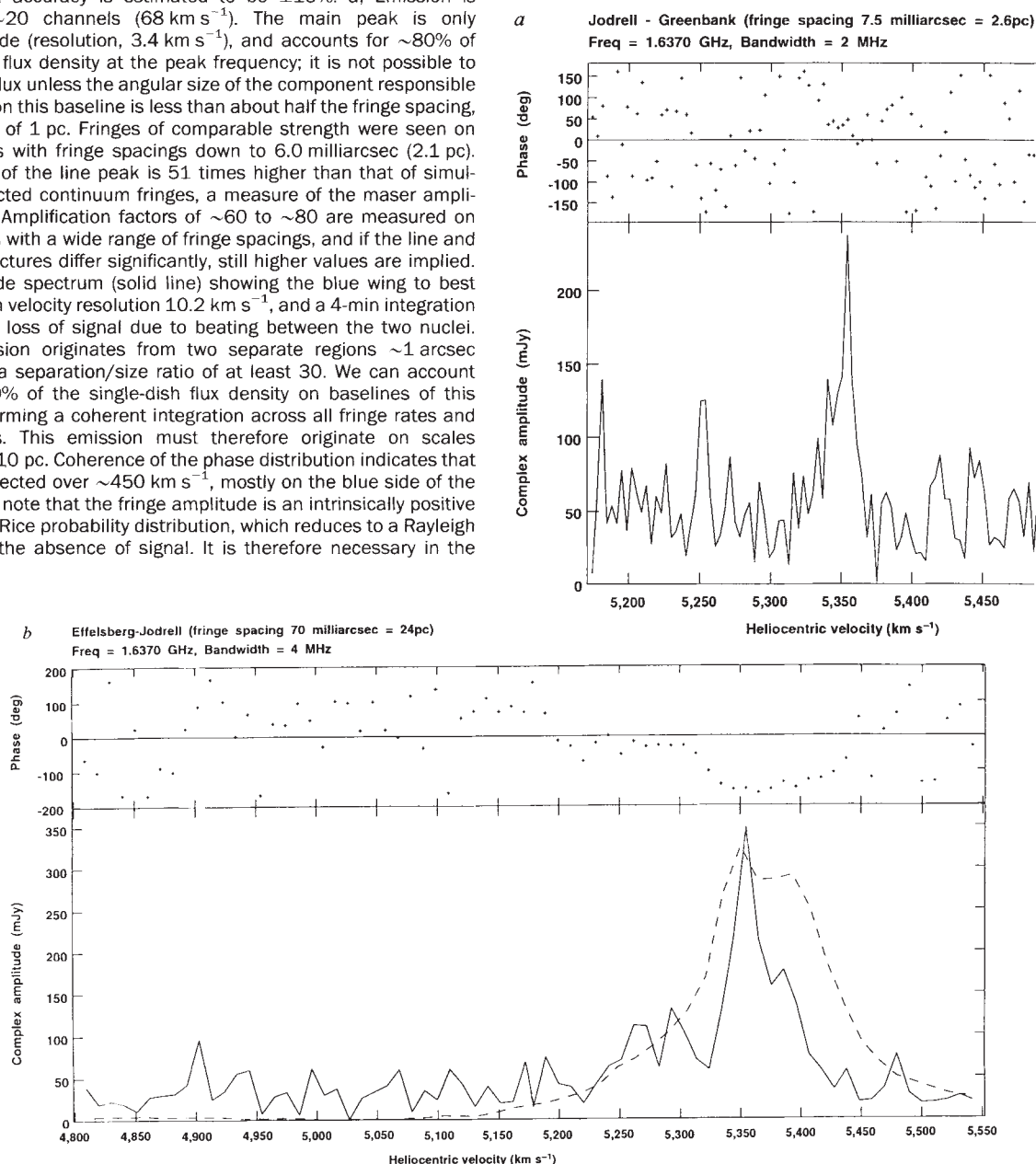
The OH megamaser phenomenon was discovered³ in 1982 in the ultraluminous infrared galaxy Arp 220 (far-infrared luminosity $L_{\text{FIR}} \approx 10^{12} L_{\odot}$, distance $D = 76$ Mpc with $H_0 = 75 \text{ km s}^{-1} \text{ Mpc}^{-1}$, where $L_{\odot}$ is the luminosity of the Sun). Megamaser emission is characterized by relatively broad lines, frequently double-peaked, with a high ratio of 1,667 MHz to 1,665 MHz line strength⁵. The radio continuum structure of Arp 220 consists of a 1-arcsec (350 pc) double, and each component is barely resolved with ~ 0.15 -arcsec-resolution very-large-array (VLA) observations⁷. Imaging of the OH line emission showed that in Arp 220, as in other megamaser galaxies, the line emission has structure which mimics the continuum emission^{8,9}; this has led to a model in which the emission is due to a foreground screen of unsaturated low-gain masers which amplify intense background continuum emission from the galaxy^{4,6,10}. Previous OH observations¹⁰ using very-long-baseline interferometry (VLBI) revealed components of size < 30 milliarcsec associated with each continuum source, which were thought to account for only 40% of the single-dish line OH flux. These were interpreted as amplified images of relatively strong, but undetected, VLBI-scale continuum components, consistent with

the basic megamaser picture. Radii of 100–300 pc for the distance of the clouds from the nucleus are suggested¹¹, though a high-velocity outflow may originate from radii as small as 10 pc (refs 4, 12). The powerful 50–120 μm infrared radiation field of the nuclear region is thought to pump the masers. Among the 50 or so known megamasers, a rough dependence of the OH luminosity $L_{\text{OH}} \propto L_{\text{FIR}}^2$ has been established^{4,5}. The OH gas may trace very high-density regions⁴ (molecular hydrogen number density $n_{\text{H}_2} = 10^5\text{--}10^7$).

FIG. 1 Fringe amplitude and phase as a function of heliocentric velocity, relative to the OH transition at 1,667.359 MHz, for two representative baselines. The data analysis, including fringe searching, was performed using the NRAO AIPS software package. In interferometry, weak signals are most easily recognized by a non-random phase distribution; in the displayed spectra weak detections are evident through non-random channel-to-channel phase distributions in several places, despite low amplitudes. Calibration of the visibilities was performed using the gain and system-temperature measurements supplied by each observatory. The calibration was refined using continuum observations of barely-resolved extragalactic calibrator sources 1611+343 and 0235+164, under the assumption of flat visibility curves on the longer baselines. The calibration accuracy is estimated to be $\pm 15\%$. a, Emission is detected in ~ 20 channels (68 km s^{-1}). The main peak is only $\sim 10 \text{ km s}^{-1}$ wide (resolution, 3.4 km s^{-1}), and accounts for $\sim 80\%$ of the single-dish flux density at the peak frequency; it is not possible to see this much flux unless the angular size of the component responsible for the fringes on this baseline is less than about half the fringe spacing, or of the order of 1 pc. Fringes of comparable strength were seen on other baselines with fringe spacings down to 6.0 milliarcsec (2.1 pc). The amplitude of the line peak is 51 times higher than that of simultaneously-detected continuum fringes, a measure of the maser amplification factor. Amplification factors of ~ 60 to ~ 80 are measured on other baselines with a wide range of fringe spacings, and if the line and continuum structures differ significantly, still higher values are implied. b, A 4-MHz wide spectrum (solid line) showing the blue wing to best advantage, with velocity resolution 10.2 km s^{-1} , and a 4-min integration time to reduce loss of signal due to beating between the two nuclei. The line emission originates from two separate regions $\sim 1 \text{ arcsec}$ apart^{9,11}, with a separation/size ratio of at least 30. We can account for at least 70% of the single-dish flux density on baselines of this length by performing a coherent integration across all fringe rates and all frequencies. This emission must therefore originate on scales smaller than $\sim 10 \text{ pc}$. Coherence of the phase distribution indicates that emission is detected over $\sim 450 \text{ km s}^{-1}$, mostly on the blue side of the main peak. We note that the fringe amplitude is an intrinsically positive quantity with a Rice probability distribution, which reduces to a Rayleigh distribution in the absence of signal. It is therefore necessary in the

During a continuum global VLBI experiment on 19 and 20 September 1992, data on Arp 220 were obtained using the Effelsberg (100-m), Jodrell Bank (76-m), Madrid (70-m) and Greenbank WV (43-m) telescopes. By chance, the redshifted 1,667 MHz OH maser main line fell within the 56 MHz passband of the 28-channel VLBI observations in a significant subset of these data, allowing us to investigate the properties of the maser at unprecedented angular resolution. Many detections of the maser were made, even on the longest baselines, often with

low signal-to-noise case ($5,000 \text{ km s}^{-1}$ to $5,200 \text{ km s}^{-1}$) to subtract in quadrature a noise component of $\sim 25 \text{ mJy}$ in order to estimate the correct fringe amplitude of $30 \pm 10 \text{ mJy}$. The dashed line is the Arecibo single-dish spectrum taken from Baan, Wood and Haschick³, presented for comparison purposes. It is intended to illustrate the velocity registration of certain features only, and because of the sharply differing nature of the data sets (one a real quantity, the other a complex quantity with complications due to fringe rate resolution), should not be compared in detail, especially in the line wings. In particular, note that any single slice through our dataset in fringe rate, such as this Figure, does not represent the total OH luminosity on these scales, thus a direct comparison with the single-dish flux density is not possible from this figure.



simultaneous detection of continuum fringes. It was, however, possible to achieve high spectral resolution for only a small quantity of data, because most of the original data tapes had been recycled after completion of the continuum-mode correlation.

High-resolution spectra for integrations of several minutes on two representative baselines, showing the amplitude and phase of the complex visibility as a function of heliocentric velocity, are shown in Fig. 1*a, b*. The line peak as seen in single-dish observations³ is dominated by a feature of dimension not exceeding 1 pc. By integrating the data across all fringe rates and frequencies, we find that $\sim 70\%$ of the total single-dish OH emission occurs on linear size scales of 10 pc or less along the line-of-sight to the compact continuum sources. By contrast, the continuum emission is dominated by a diffuse component of linear size ~ 100 pc (refs 7, 9, 13, 14), and only 1.5–3% of the flux density of ~ 300 mJy is in compact features detected on our VLBI baselines. Each component of the 1-arcsec double structure in Arp 220 displays the line and continuum characteristics described above. Continuum and line fringes from the western nucleus are simultaneously detected in continuum-mode correlations of two, unconfused long baselines. The line and continuum fringe phases on these baselines differ by only 20° and 40° respectively, demonstrating that at least for the western nucleus, the parsec-scale line and continuum features are positionally coincident to within ~ 0.2 pc.

The spectra given in Fig. 1, and other spectra in our dataset, demonstrate that the OH line emission is much more physically compact than has been previously supposed. Our VLBI OH line measurements are broadly consistent with previous results¹⁰; however, the fact that we have simultaneous continuum detections allows us to address the question of the location of the masing gas along the line-of-sight to the nuclear continuum sources. This is because we can directly measure amplification factors on various size scales by comparison of simultaneous line and continuum correlated flux densities on various baselines. The maser amplification factor within ~ 10 pc of the VLBI continuum emission is measured to be ≥ 60 , and the line emission from these scales accounts for $\geq 70\%$ of the single-dish line flux associated with the source. On larger scales, the remaining 30%, combined with $\geq 97\%$ of the total continuum, yields an average maser amplification factor across the diffuse continuum not exceeding ~ 0.3 . Thus the apparent gain across the source is highly concentrated at sites of VLBI continuum emission, with mean amplification factors increasing by a factor ≥ 200 as one moves from the scale of the diffuse continuum (tens to hundreds of parsecs) to below ~ 10 pc scales. Unless our line-of-sight to this source is highly fortuitous, we must presume that the apparent gain distribution is similar when viewed from all directions, implying that the masing gas is also highly concentrated near the VLBI continuum emission, at each of the two nuclei. The probable physical extent of the bulk of the masing gas is ≤ 20 pc (radius $r \leq 10$ pc).

The OH megamaser in Arp 220 thus has a substantially different nature from that previously supposed⁵. Instead of being a large-scale phenomenon produced by molecular clouds distributed in interstellar space over a volume of the order of 10^6 pc³, in a region dominated by the nuclear stellar gravitational potential, it is a small-scale phenomenon manifested on scales which imply volumes of $\leq 10^3$ pc³, in a region where the gravitational potential is likely to be dominated by a central supermassive black hole.

To assess the far-infrared pumping photon flux through the maser, a calculation involving the isotropic luminosity of the maser, pump efficiencies, velocity dispersions and other variables is necessary (see, for example, Reid and Moran¹⁵). We have performed such a calculation under reasonable assumptions (C.J.L., P.J.D., H.E.S. and C.J.L., manuscript in preparation), and find that the maser is probably pumped by a compact, warm ($r \leq 30$ pc, temperature $T \geq 150$ K) infrared source hidden from

direct view by cooler dust which reprocesses the radiation into the observed 60–100 μ m spectral region on >100 pc scales. We calculate that an infrared source of this size and temperature must supply a fraction $0.01/\epsilon$ of the total luminosity of Arp 220 (where ϵ is the pump photon efficiency) in order to power the observed OH emission. Efficiencies >0.01 are generally thought to be unlikely in discussions of maser pumping. This is a strong constraint on the location of the ultimate power source, and an indication that in Arp 220, much of the luminosity probably originates from a single central location (that is, an obscured quasar) rather than from a nuclear starburst distributed over a few hundred parsecs.

The question naturally arises as to the nature of the physical entity which contains the masing gas. Many lines of evidence indicate that the appearance of various classes of active galactic nuclei (AGN) is strongly modified by viewing angle¹⁶, and central to current models for anisotropic radiation is an obscuring dense molecular torus which forms from infalling material in the gravitational potential well of the central supermassive black hole¹⁷. The physical conditions to be expected within such a torus have been extensively modelled^{17,18}, and we can investigate the feasibility of producing the OH maser emission from such a torus.

According to these models, a typical cloud in the torus has number density $n_H \sim 10^7$, column density $\sim 10^{24}$ cm⁻², and temperature $\sim 10^3$ K. The cloud covering factor to the central AGN source is of the order of unity. Despite a high ionized fraction ($\chi_e = n_e/n_H \sim 10^{-3}$ where n_e is the number density of free electrons), caused by the penetrating hard X-ray flux from the central source, many of the model runs yield clouds transparent to free-free absorption at GHz frequencies. The predicted OH abundances relative to H₂ are typically high, $\sim 10^{-4}$, and the far-infrared optical depth τ is moderate ($\tau \approx 1$ between 50 and 100 μ m for column density $\sim 10^{24}$ cm⁻²). In a torus with these properties covering 2π sr with inner radius ~ 3 pc, $\sim 10^{39}$ OH molecules will be present, sufficient to explain the observed OH luminosity^{15,19}.

The observed line profile from a masing torus will depend not only on the gain of the masing gas along various lines of sight, but also on the strength of the background continuum available for amplification along these lines of sight. As the continuum is sharply peaked, with sub-parsec scale features, we should see a strong, relatively narrow line core with weaker broad wings. The keplerian velocities at a radius of a few parsec from a $1.5 \times 10^7 M_\odot$ black hole (where $M_\odot$ is the mass of the Sun, yielding $5 \times 10^{11} L_\odot$ at the Eddington limit for each of the two nuclei) are close to 200 km s⁻¹, so the total velocity width of the wings should be of the order of 400 km s⁻¹. This accurately describes the observed single-dish line profile³. As in any model, the narrow linewidths seen at higher resolutions in our data can be attributed to individual masing clouds along a single line-of-sight.

In summary, an AGN molecular torus model for the OH megamaser in Arp 220 fits the observations remarkably well and, in conjunction with the maser pumping constraint discussed earlier, appears to be an attractive working hypothesis. It has been pointed out¹⁸ that the torus itself is a copious source of infrared photons, and the outer parts of the torus may well represent the maser pumping source.

Arp 220 is commonly used in tests of AGN versus starburst models of ultraluminous infrared galaxies²⁰, and as such is regarded as typical of its class²¹. If our results can thus be extended to the general population of megamaser galaxies, observations of the many OH megamasers accessible to VLBI offer a powerful new probe of AGN molecular tori and the hitherto virtually unobservable interiors of the nuclei of highly obscured galaxies. Furthermore, the extensive current database on the 50 or so known OH megamasers⁵ represents fertile ground for the investigation of torus and host galaxy properties, through analysis of line ratios and profiles in the context of a new inter-

pretation. Our arguments on the pumping energetics would presumably apply to megamaser galaxies in general, strongly favouring an obscured AGN origin for the far-infrared luminosity.

The established $L_{\text{OH}} \propto L_{\text{FIR}}^2$ relationship is consistent with this new picture. Evidence is available that the continuum VLBI-scale radio power L_{VLBI} is proportional to L_{FIR} in far-infrared galaxies²². The continuum luminosity on parsec scales available for amplification by OH gas in the torus is L_{VLBI} , different by a large factor from the continuum luminosity on larger scales used in previous arguments of this type. At the same time, the flux of infrared pumping photons is also proportional to the far-infrared luminosity, so that $L_{\text{OH}} \propto L_{\text{FIR}} L_{\text{VLBI}} \propto L_{\text{FIR}}^2$, as observed.

Because a torus model predicts OH megamaser angular sizes that are resolvable only with VLBI, reports of apparent spatial

velocity gradients on larger scales^{19,23} seem to conflict with such a model. However, ultraluminous infrared galaxies are strongly associated with galactic merger events, and the presence of two nuclei, each with its own masing torus, may be common (compare the early VLA observations⁸ of Arp 220). We support the suggestion⁵ that the typical double-peaked line profile in OH megamasers is also a consequence of dual nuclei in merging systems, which may be very close together spatially.

Finally, Fig. 1b shows evidence for significant emission on the blue side of the main peak, out to $\sim 5,000 \text{ km s}^{-1}$. The flux density in this region is estimated to be $30 \pm 10 \text{ mJy}$, which when compared to a single-dish spectrum taken only 4.5 years earlier¹² suggests variability, but we hesitate to interpret it as such because of baseline subtraction and calibration uncertainties in the single-dish data. $\square$

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Density of comet Shoemaker–Levy 9 deduced by modelling breakup of the parent ‘rubble pile’

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FOR a week beginning 16 July 1994, fragments of comet Shoemaker–Levy 9 will collide with Jupiter each day. Although the fragments are probably smaller than originally estimated¹, the impacts may nevertheless have observable consequences that will provide valuable insight into the properties of comets and the dynamics of planetary atmospheres. Interpretation of these observations will depend sensitively on parameters such as the mass, density and overall structure of the fragments. To deduce some of these parameters, we have simulated the event that created the fragments—the passage of the parent comet through the tidal field of Jupiter in 1992. Modelling the comet as a strengthless aggregate consisting of a large number of grains, we find that the tidally disrupted body condenses rapidly into clumps, driven by their self-gravity. Formation of a fragment chain resembling Shoemaker–Levy 9 occurs for a narrow range of the simulated comet’s bulk density, $0.3\text{--}0.7 \text{ g cm}^{-3}$. A chain of ~ 20 similar-sized fragments matching observations is obtained for a non-rotating parent comet of 1.5 km diameter and bulk density 0.5 g cm^{-3} , suggesting that the clusters will each liberate $\sim 10^{27} \text{ erg}$ on impact. A slightly larger initial density leads to significant mass variation among the clusters and the possibility of a few $\sim 10^{28}\text{-erg}$ events.

Two years ago, comet Shoemaker–Levy 9 traversed the Roche limit of Jupiter over the course of a few hours, achieving a closest approach of $\sim 94,000 \text{ km} = 1.31 R_J$ from the planet centre, where R_J is the jovian radius (P. W. Chodas and D. K. Yeomans, unpublished results) in a captured orbit with eccentricity $e \approx 0.996$. It is widely believed that this tidal encounter created the “string of pearls” now predicted to strike the planet^{2,3}. To eliminate one possibility, we first show that a homogeneous brittle object of arbitrarily low but finite strength cannot be tidally disrupted into the observed number of pieces along the inbound trajectory of Shoemaker–Levy 9. For such an object to disrupt, not only must tidally-induced stress exceed self-gravitational compression⁴, but material strength (however small) must also be overcome: the body must fracture. Simple fracture mechanics shows that both criteria are met in a body with strength only if its density is unreasonably low.

A crack propagates at about half the sound speed⁵ through a brittle object subject to uniform tension, and relieves stresses across a $\sim 1\text{-km}$ ball of ice or snow (for instance) in seconds. The rate of increase of the tidal stress, as the body approaches the planet, is about four orders of magnitude lower, as the distance R to Jupiter decreases by at most $\sim 1 R_J$ per hour. Fracture is therefore quasi-static, with a single crack relieving stresses faster than they accrue⁵. The crack may stall if stress drops rapidly, but being the weakest region, it will finish growing before new cracks develop. Once the comet breaks in two, tidal stress $\sigma_T \propto d^2/R^3$ drops below the fracture threshold in each half, where d is the new, smaller size and R is the distance to Jupiter. The halves split into four if σ_T exceeds material strength at a closer distance. Tidal stress then drops again for the even smaller size, and so on, in sequential bifurcation.

The final fragments are approximately one-quarter of the original size, depending on geometry, if more than 20 pieces form by such a hierarchy of fracture. If strength is uniform, then the first crack must begin more than $\sim 4^{2/3} = 2.5$ times the perijove distance, or $\sim 3.3 R_J$. But splitting at $3.3 R_J$ is impossible even for strengthless objects with density $\rho \geq 0.05 \text{ g cm}^{-3}$,

TABLE 1 Outcomes for tidal disruption of aggregates

Initial diameter (km)	Bulk density (g cm^{-3})	Number of grains	Grain diameter (m)	Initial rotation	Number of clumps*	Remarks
1.5	0.1	500	170	None	Diffuse	Subtle structure; four to five dense regions
1.5	0.3	500	170	None	Diffuse	Two to three incipient clumps; mostly stray grains
1.5	0.5	500	170	None	15+	Uniform clumping (Figs 1, 2 and 3b)
1.5	0.7	500	170	None	12+	Two clumps contain 40% of mass
1.5	1.0	500	170	None	3+	Central clump contains 80% of mass
1.5	2.0	500	170	None	1	Central clump contains 95% of mass
1.5	0.5	21	480	None	10	Single grains count as clumps in this case only
1.5	0.5	100	290	None	9	Coarse irregular clumping
1.5	0.5	1,000	136	None	20+	Uniform clumping (Fig. 3c)
1.5	0.5	2,000	108	None	20+	Uniform clumping (Fig. 3d-f)
4.0	0.5	2,000	108	None	17+	Similar to above, only larger (Fig. 1)
1.5	0.5	500	170	9 h†	7+	Broadly scattered grains; stalled clumping
1.0	1.0	500	170	9 h‡	12+	Prograde; mimics no. 3 in clumping and length (Fig. 1)
1.5	0.5	500	170	9 h‡	2	Retrograde; central clump contains 90% of mass

* A clump is a gravitational agglomeration of several grains which remains constant to late time. In the case $n_c = 21$ we count solitary grains as clumps, whereas in all other cases we require three or more grains per clump. As the grains become more numerous, clump morphology becomes obvious; see Fig. 3e for example. In all cases we look at detailed zooms at numerous timesteps to determine clumps.

† Axis of rotation in the plane of the orbit.

‡ Axis of rotation parallel to the axis of the orbit.

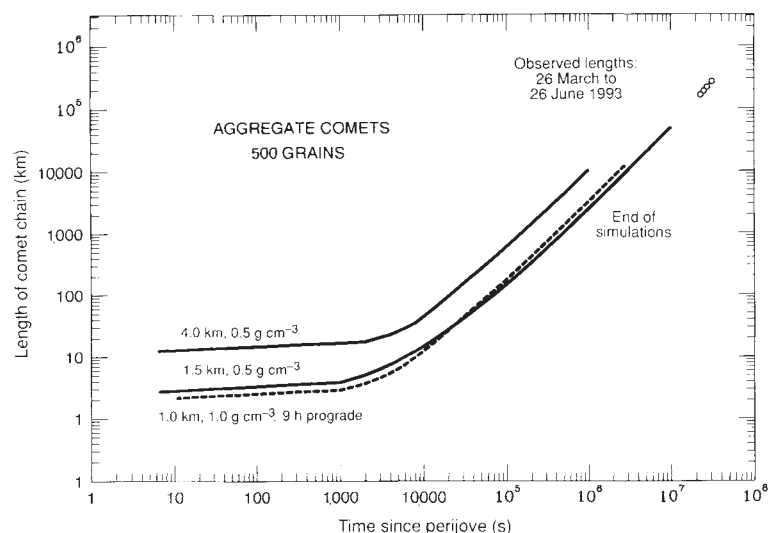
because self-gravity exceeds the tides. This density threshold is also observed in smooth-particle hydrodynamics (SPH) models in which lithostatic overburden is added to a vanishingly small material strength⁶. This density is near the absolute lower limit determined for Halley's nucleus⁷. If growing cracks stall, if smaller pieces are stronger than the whole, or if the final fragments formed earlier than perijove (all three are likely), then fracture must begin even farther from Jupiter, requiring even lower densities. A uniform body of any realistic density could not have been broken into ~ 21 pieces by the tides, no matter how low its strength. It must have been broken already.

Consequently, the comet was presumably either a 'rubble pile'⁸ to begin with, or else a solid body (or weakly cemented aggregate) shattered by impact during the inbound ring-plane crossing (see below). Scotti and Melosh² propose a strengthless agglomeration of the ~ 21 objects seen today, and Melosh and Schenk⁹ in a related paper consider that kilometre-sized 'cometesimals' may be the fundamental sub-structure of comets, based on the uniform sizes in crater chains on Ganymede and Callisto. Their proposed cometesimals are, however, much larger than the grain size predicted by accretion models¹⁰. Here we present an alternative explanation which also assumes a 'rubble pile', but does not require large or uniform grains.

We simulate the comet as a fixed number n_c of spheres in hexagonal closest packing. The bulk comet is 27% less dense than the individual grains due to voids. Self-gravity is calculated between grains, and a radial restitutive force increases with depth of penetration when they intersect. Cohesion between grains is zero and friction is ignored. We send these strengthless clusters on the comet's 1992 orbit past Jupiter and observe their fate: the differential pull of gravity from the planet (the tide) rips the comet apart, while self-gravity resists disruption and causes clump instabilities among the grains. Chandrasekhar¹¹ predicted such instabilities for infinite cylinders of given density and radius, but these parameters change rapidly in time and position for the Shoemaker-Levy 9 chain. To calculate the orbit we assume perijove is $1.3R_J$ and $e = 1$. Jupiter is modelled as a spherical mass, and the Sun is not included. Although the Sun is responsible for the comet crashing into Jupiter, this arises from a third-body perturbation of $\sim 1R_J$ over the entire orbit. The Sun's tide—the contribution relevant to our analysis—is 0.2 times the jovian tide at apojove and less than 0.1 times the jovian tide when averaged over the orbit. This introduces an uncertainty of $\sim 10\%$ in chain length, but does not affect disruption and clumping, which take place very near Jupiter.

We constrain the diameter of the parent comet by starting

FIG. 1 N -body simulations produce chains of fragments whose lengths are determined by a number of factors, including perijove distance, parent body size and initial rotation. This graph shows the chain length of three strengthless aggregates as a function of time. Open circles near $\sim 3 \times 10^7$ s (~ 1 year) are observations². Two solid lines are non-rotating comets with density 0.5 g cm^{-3} , and the dotted line is a prograde rotating comet with density 1.0 g cm^{-3} . This 9-h rotating comet mimics the larger, less dense comet not only in terms of chain length but also in terms of clump distribution. The shortest stable rotation period for a strengthless sphere of bulk density ρ (in g cm^{-3}) is $P_{\min} = 3.3/\sqrt{\rho}$ hours, so that smaller, more rapidly rotating prograde parent comets need to be considered. Retrograde-rotating parents form a massive central clump (see text) and can therefore be ruled out. The non-rotating parent therefore represents an upper limit to the total cometary mass, containing nearly twice the mass of the smaller prograde parent in spite of its lower density.



with an initial guess and evolving it. The length of the debris chain is then compared with observations (Fig. 1). A 1.5-km-diameter non-rotating comet with a bulk density of 0.5 g cm^{-3} yields a chain of the required size. (This density is preferred because it also produces the required number of clumps; see below.) The $4/3$ exponent of chain length versus time agrees with Sridhar and Tremaine's formal analysis¹². Scotti and Melosh² derive a diameter $\sim 1.6 \text{ km}$ using a related method, assuming an earlier perijove estimate of $1.57 R_J$. They neglect self-gravity but include all nine planets and the Sun.

Prograde rotation produces a longer chain for a given initial size, so that smaller comets can also fit the data. A 1.0-km-diameter comet of bulk density 1.0 g cm^{-3} with rapid (9 h) prograde rotation (Fig. 1) yields a chain length similar to the 1.5-km non-rotator with similar clumping. Retrograde rotation, by contrast, causes a massive central clump not evident in the Shoemaker-Levy 9 chain. Large central craters in some chains on Ganymede and Callisto might correspond to retrograde disrupted comets, but they may also correspond to density fluctuations in the parent, or an undivided nucleus. Rotation orthogonal to the orbit causes diffusive scattering of grains and can hinder clumping.

Bulk density is constrained by requiring clumping to agree with observations. Insufficient coagulation occurs when $\rho \leq 0.3 \text{ g cm}^{-3}$, and a single massive clump forms when $\rho =$

1.0 g cm^{-3} (Table 1). The range of allowed densities is narrow; evenly-spaced, similar-sized clumps result when $\rho \approx 0.5 \text{ g cm}^{-3}$ for this orbit. It is possible, however, that only a few major clumps really exist in the Shoemaker-Levy 9 chain, and that many of the visible objects possess little mass. Strongly heterogeneous mass distributions result in our model for initial bulk densities of $\sim 0.6\text{--}0.7 \text{ g cm}^{-3}$. Heterogeneity is suggested by the continuing disappearance of objects in the chain, in which case a couple of $\sim 10^{28}$ -erg events might occur, with the remainder being comparatively small. Impact photometry will distinguish between these scenarios and place a final constraint on the parent comet's density.

Figure 2a shows a 1.5-km-diameter 500-grain comet of bulk density $\rho = 0.5 \text{ g cm}^{-3}$ evolving in orbit past Jupiter. This view greatly exaggerates the size of the fragment chain. A close view at perijove (Fig. 2b) shows the tidal strain. Gravitational instability condenses the extending mass into clumps over the course of the next several hours (Fig. 2c, d). Roughly the same number of clumps form for 600-m, 1.5-km and 4-km comets, so this density seems not to depend strongly on size. The final mass distribution in the chain is determined relatively early, and after a few days (Fig. 2e) the clumps follow independent trajectories.

Provided that grain size is much smaller than the wavelength of the clumping instability, these results are robust to changes in n_c . Figure 3 shows the comet at $18 R_J$ (between the orbits of

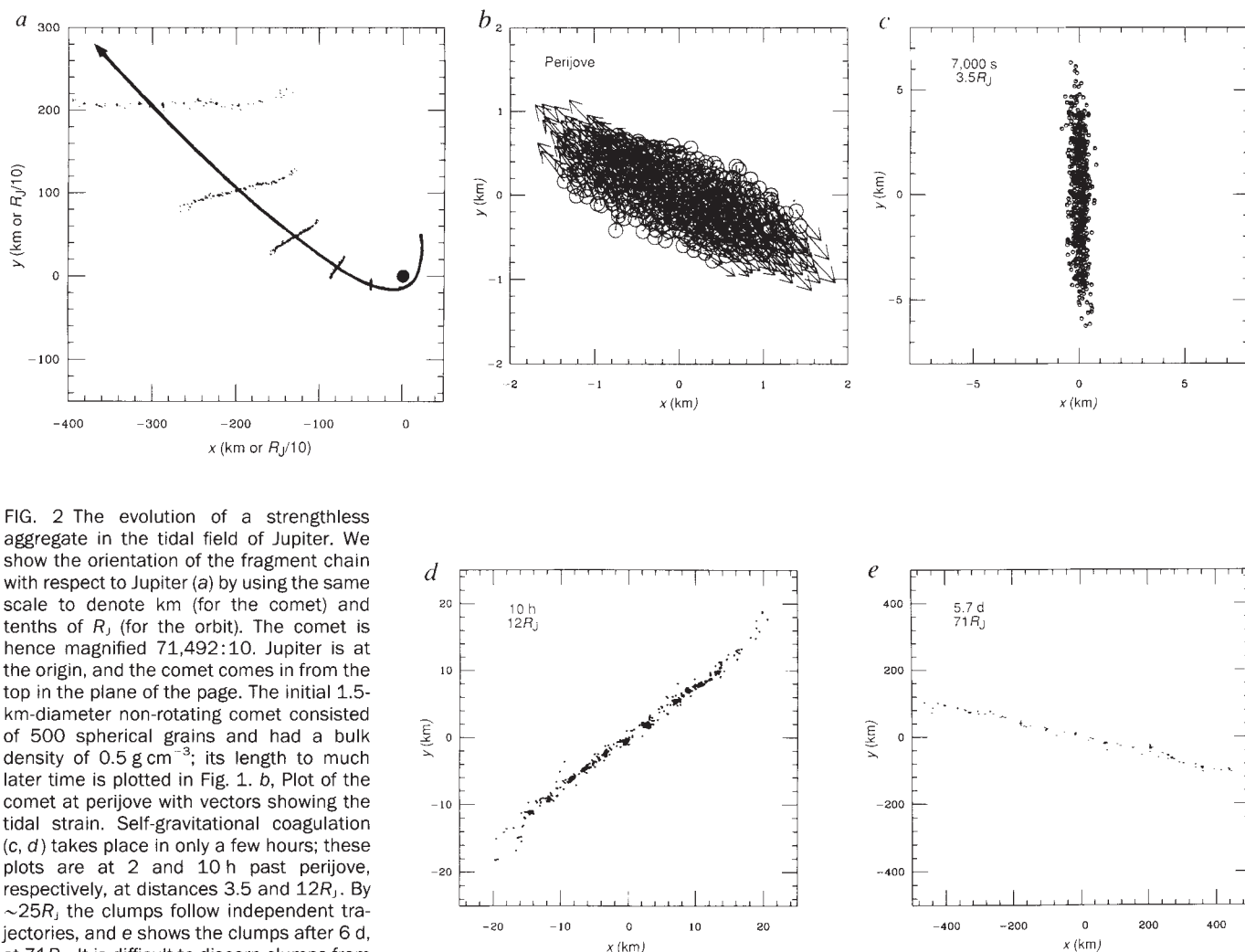


FIG. 2 The evolution of a strengthless aggregate in the tidal field of Jupiter. We show the orientation of the fragment chain with respect to Jupiter (a) by using the same scale to denote km (for the comet) and tenths of R_J (for the orbit). The comet is hence magnified 71,492:10. Jupiter is at the origin, and the comet comes in from the top in the plane of the page. The initial 1.5-km-diameter non-rotating comet consisted of 500 spherical grains and had a bulk density of 0.5 g cm^{-3} ; its length to much later time is plotted in Fig. 1. b, Plot of the comet at perijove with vectors showing the tidal strain. Self-gravitational coagulation (c, d) takes place in only a few hours; these plots are at 2 and 10 h past perijove, respectively, at distances 3.5 and $12 R_J$. By $\sim 25 R_J$ the clumps follow independent trajectories, and e shows the clumps after 6 d, at $71 R_J$. It is difficult to discern clumps from stray grains at late time from these images alone, they are two-dimensional projections of three-dimensional data.

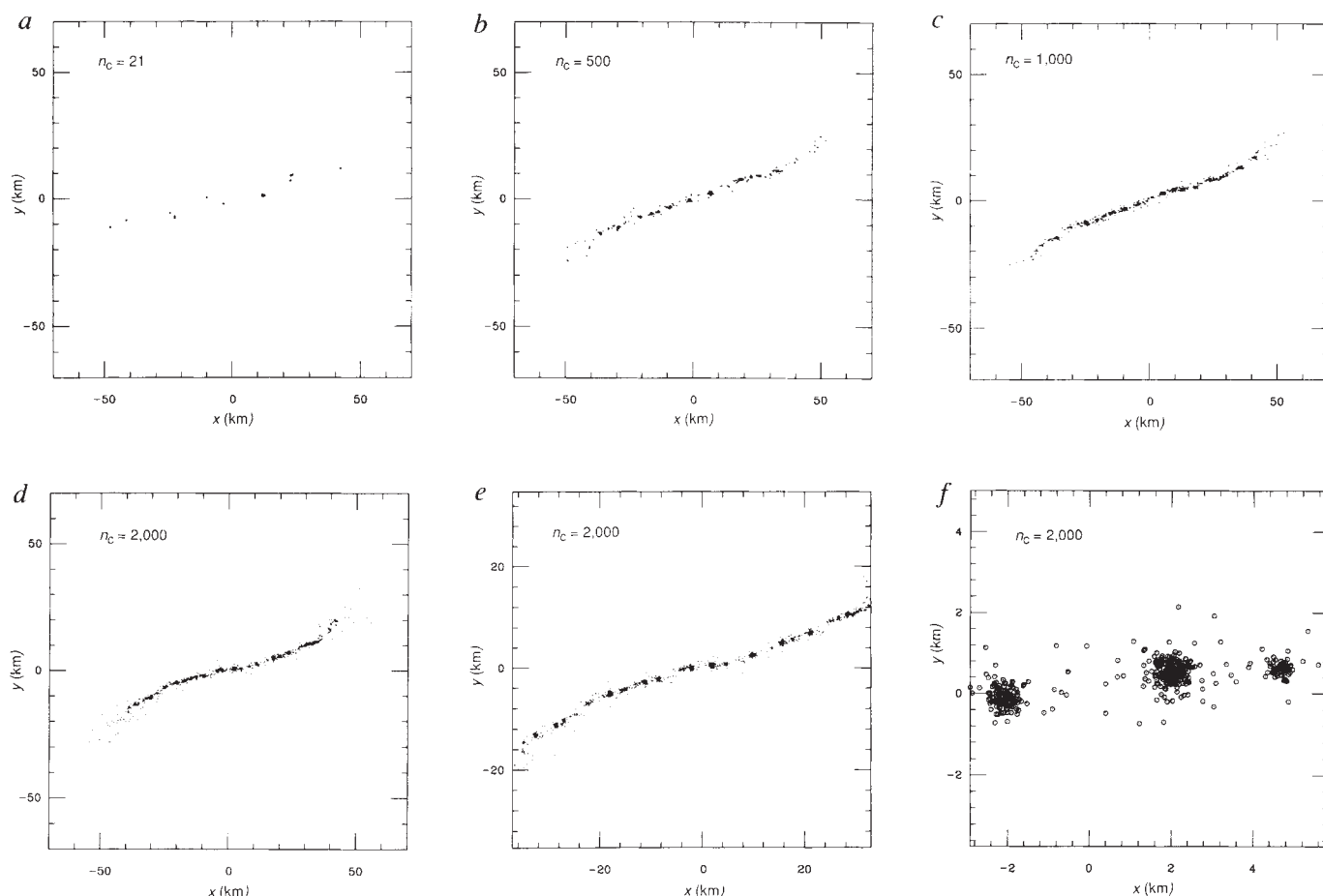


FIG. 3 *a-f*, Outcomes for the tidal disruption and gravitational coagulation of 1.5-km-diameter 0.5 g cm^{-3} aggregates. The figures vary in grain size from coarse to fine, $n_c = 21, 500, 1,000$ and $2,000$ (shown in each panel), with corresponding grain diameter 480 m, 170 m, 136 m and 108 m. All are shown 18 h after perijove, $18R_J$ from Jupiter, between the orbits of Ganymede ($15R_J$) and Callisto ($26R_J$). See Fig. 2*a* for a schematic of the orbit. The 21-grain comet (*a*) forms only 10 clumps, as several grains merge. For 500 or more grains, however, behaviour is similar regardless of grain size (*b-d*). It is difficult to distinguish clumps from stray grains in these figures; to compile Table 1 we zoomed in on candidate clumps at a later time. Zooms on the $n_c = 2,000$ chain (*e, f*)

Ganymede and Callisto) for $n_c = 21, 500, 1,000$ and $2,000$. Chain length is about the same in each case, but clumping is more uniform for fine-grained comets. Note that 21 grains of this density coagulate into ~ 10 clumps instead of moving on independent paths. We find that 21 grains move as 21 independent objects only if the grain density does not exceed 0.1 g cm^{-3} (assuming no rotation). This fixes an upper limit to density in the large-cometesimal model^{2,9}. Weidenschilling¹⁰ suggests a fundamental grain size of a few tens of metres based on Goldreich-Ward condensation in the outer nebula; we are evolving a system of 20,000 grains to test this idea. Based on present results, we expect more extensive debris trails before and after the major clumps, as smaller grains are more easily scattered by gravitational interactions. The smallest grains considered here (Fig. 3*f*) are 108 m across. Whereas ~ 200 m is the largest grain size which leads to uniform clumping in our model, we have not yet encountered a lower limit.

Do these results imply that all comets are strengthless rubble piles? We note that the inbound comet crossed the equatorial plane of Jupiter at $1.6 \pm 0.2R_J$ (ref. 13). The main ring of Jupiter lies within this error bar. According to scaling laws¹⁴, a 1.5-km-diameter sphere of ice can be catastrophically destroyed on

show individual clumps quite clearly. Our model lacks damping; we probably overestimate the percentage of stray grains. In any event a re-assembled body exists within each clump, not fundamentally different from the original strengthless parent comet, although lower in overall density. The debris chains shown here are somewhat curved, but they straighten out as they lengthen. Net curvature out of the orbital plane, in the z -direction, occurs only for rapidly rotating inclined parent comets. Therefore this debris projects as a straight line in the direction of its motion and would produce a linear crater chain were it to strike a fixed flat surface.

impact with a single 1.5-m particle at 50 km s^{-1} . If the optical depth of the ring is $\sim 10^{-6}$ in such objects, the comet is likely to hit about one of them. Such a violent impact can create the rubble pile *in situ*, particularly for bodies already weaker than pure ice. The bonds of a weakly cemented aggregate, for example, could easily be broken by this shock, or by millions of hypervelocity impacts with smaller grains. Ring-particle collisions before perijove are more probable for comets striking Ganymede and Callisto, which orbit in the equatorial plane. This idea is consistent with the production of crater chains, particularly if the jovian rings have in the past been more substantial. Passage through the Roche limit of a giant planet can be hazardous in more ways than one. $\square$

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Square chemical waves in the catalytic reaction $\text{NO} + \text{H}_2$ on a rhodium(110) surface

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IT WAS realized as early as 1906¹ that coupling between an autocatalytic reaction and the diffusion of the autocatalytic component can give rise to a propagating reaction front—a chemical wave. Chemical waves have been studied intensively in fluid-phase reaction–diffusion systems², but in recent years a variety of spatio-temporal patterns has also been observed for oscillatory reactions on single-crystal surfaces^{3,4}. One important new aspect that has been introduced by these studies is that of anisotropic diffusion, as a consequence of the fixed surface geometry on which the diffusion of the adsorbed particles takes place. Here we report the observation of a transition from elliptical to square-shaped concentric chemical waves in the reaction of NO and H_2 on a rhodium(110) surface. The elliptical pattern is characteristic of simple anisotropic diffusion, but we attribute the origin of the square pattern to a state-dependent anisotropy—that is an anisotropy that varies along the wave profile as changes in the adsorbate coverage generate different reconstructions of the substrate structure. This interplay between diffusional anisotropy and the state of the system can be expected to be quite general and to give rise to new varieties of oscillatory patterning.

The reaction of NO and H_2 , yielding mainly N_2 and H_2O as products, proceeds on Rh(110) by dissociation of molecularly adsorbed NO into atomic nitrogen and oxygen; this is followed

by recombination of the dissociated reactants to give products^{5,7}. In the experiments reported here we employed a Rh(110) single crystal of $\sim 0.3 \text{ cm}^2$ surface area as the catalyst, and operated under isothermal conditions at a pressure of 10^{-6} – 10^{-4} mbar using the ultra-high-vacuum chamber as a gradient-free flow reactor. The laterally varying adsorbate concentration was imaged under reaction conditions by photoemission electron microscopy (PEEM)⁸. This method, which yields a spatial resolution of $\sim 1 \mu\text{m}$, is based on the principle that the yield of photoelectrons depends sensitively on the local work function as one illuminates the sample with photons from a deuterium discharge lamp. Under reaction conditions at temperature $T > 500 \text{ K}$, atomic nitrogen (N_{ad}) and oxygen (O_{ad}) are the main species present on the surface^{5,6}; because O_{ad} causes a stronger work function increase than N_{ad} , we image essentially the oxygen concentration in PEEM. Oxygen-rich areas appear dark, and oxygen-deficient areas bright.

In the 10^{-6} to 10^{-4} mbar range investigated here, the reaction system displayed excitability. Target patterns were initiated in a typical procedure by first raising the partial pressure of hydrogen (p_{H_2}) to 1.2×10^{-4} mbar, then decreasing it to a value where the nucleation of reaction fronts takes place (this was carried out at a constant partial pressure of NO, p_{NO} , of 1.6×10^{-6} mbar). At $T = 540 \text{ K}$, elliptical target patterns are observed (Fig. 1a). The elliptical shape of the target patterns results from anisotropic front propagation velocity which exhibits values of $2.6 \mu\text{m s}^{-1}$ and $0.8 \mu\text{m s}^{-1}$, respectively, along the two main crystallographic directions.

On raising the temperature to 595 K, the shape of the target patterns changes continuously as the vertical and horizontal parts of the pattern elongate. Finally, one observes the nucleation of almost square patterns, as shown in Fig. 1b and c. The sides of the squares are orientated along the main crystallographic directions of Rh(110). As more and more of the waves are emanated, the travelling white bands start to break into small pieces forming a complex pattern, the initial stage of which is already visible in Fig. 1c.

For an explanation of the observed phenomena one has to look at the effect of the substrate geometry on surface diffusion. Both atomic oxygen and nitrogen induce reconstructions of the Rh(110) surface; however, these reconstructions are of a different type, as illustrated by the structural models shown in Fig. 2. Oxygen induces reconstructions of the missing-row type, of

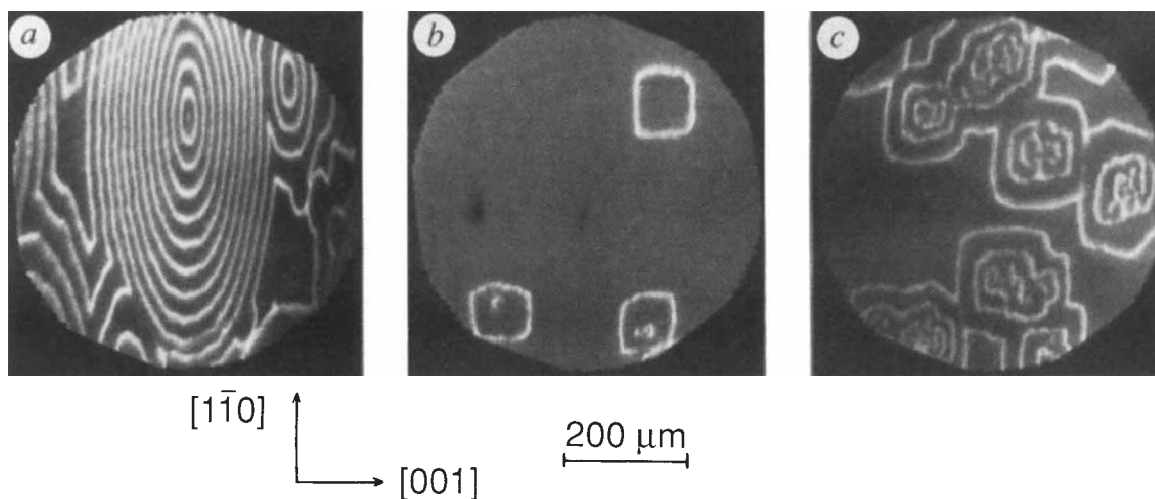
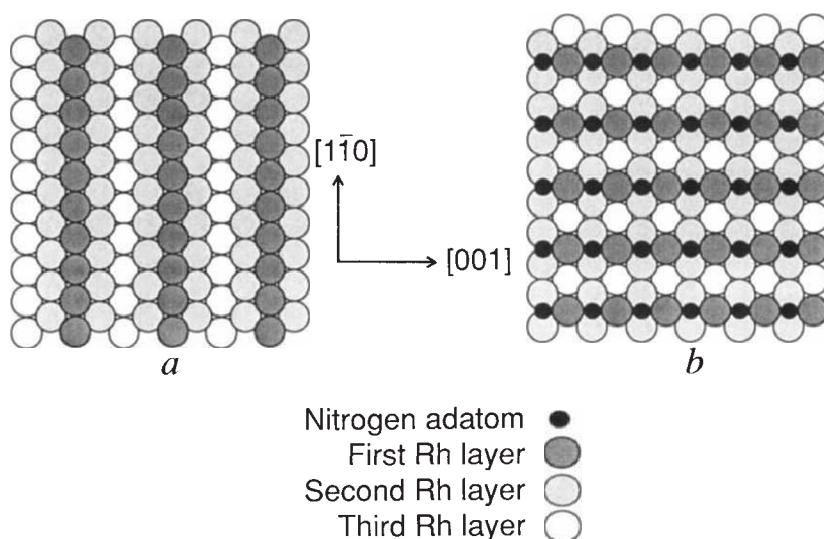


FIG. 1 Photoemission electron micrographs showing different geometries of target patterns in the $\text{NO} + \text{H}_2$ reaction on Rh(110). a, Elliptical target pattern. Experimental conditions: $T = 540 \text{ K}$, $p_{\text{NO}} = 1.6 \times 10^{-6} \text{ mbar}$, $p_{\text{H}_2} = 1.8 \times 10^{-5} \text{ mbar}$. b, Nucleation of a square-

shaped target pattern. Experimental conditions: $T = 595 \text{ K}$, $p_{\text{NO}} = 1.6 \times 10^{-6} \text{ mbar}$, $p_{\text{H}_2} = 5 \times 10^{-6} \text{ mbar}$. c, Square-shaped target pattern recorded 60 s after nucleation.

FIG. 2 Structural models of the adsorbate-induced reconstructions which are relevant in the $\text{NO} + \text{H}_2$ reaction on $\text{Rh}(110)$ (after ref. 6). a, Model of the 1×2 missing-row reconstruction, which is the substrate geometry proposed for the mixed $c(2 \times 4)$ -N,O adlayer. The adatoms are not shown. b, Model of the (2×1) -N reconstruction.



which the simplest example is the 1×2 reconstruction shown in Fig. 2a (refs 9, 10). The same substrate geometry has been proposed for the mixed $c(2 \times 4)$ -N,O overlayer which has been associated with the dark area in the PEEM images^{6,11}. In contrast, the white area in the PEEM images has been identified as a 2×1 -N reconstruction, a model of which is shown in Fig. 2b (ref. 5).

Surface diffusion of a mobile adsorbate such as adsorbed hydrogen or NO will be fast along the troughs of the 1×2 reconstruction (Fig. 2a), but slow perpendicular to them. Consequently, one expects an anisotropy in the front velocity which is fast along the $[1\bar{1}0]$ direction, and this is what is seen in Fig. 1a. In comparison to the 1×2 geometry, the rows of the 2×1 -N reconstruction (Fig. 2b) are rotated by 90° . This has the consequence that as the reaction front propagates, the anisotropy changes by 90° . It is this change in anisotropy which we think is responsible for the unusual square-like geometry of the target pattern observed in this reaction system. Without this state-dependent anisotropy of surface diffusion, scaling arguments would always predict elliptical patterns, provided that a single diffusing species is involved¹².

Figure 3 shows the results of a particularly interesting experiment which demonstrates the influence of p_{H_2} on the anisotropy and excitability of the system. After initiating the square-like

target pattern (Fig. 3a), p_{H_2} was reduced from 5×10^{-6} to 4.5×10^{-6} mbar. This caused the horizontal parts of the pattern to disappear while the vertical parts remained, as shown in Fig. 3b. When p_{H_2} was subsequently raised to 5×10^{-6} mbar, the free ends of the vertical bars curled in to form spirals with sharp corners (Fig. 3c).

With respect to the experiments shown above, we cannot completely rule out a possible influence of surface defects such as polishing scratches. But because repetition of the experiments with a second $\text{Rh}(110)$ single crystal led to the same results, and because the square-like target patterns are orientated along crystallographic directions, such a possibility appears to be rather unlikely. It has recently been shown¹² theoretically that wave patterns with sharp edges can form if the front velocity exhibits such a complex anisotropy; the novel phenomenon described here should stimulate both theory and experiment in the field of spatiotemporal pattern formation. □

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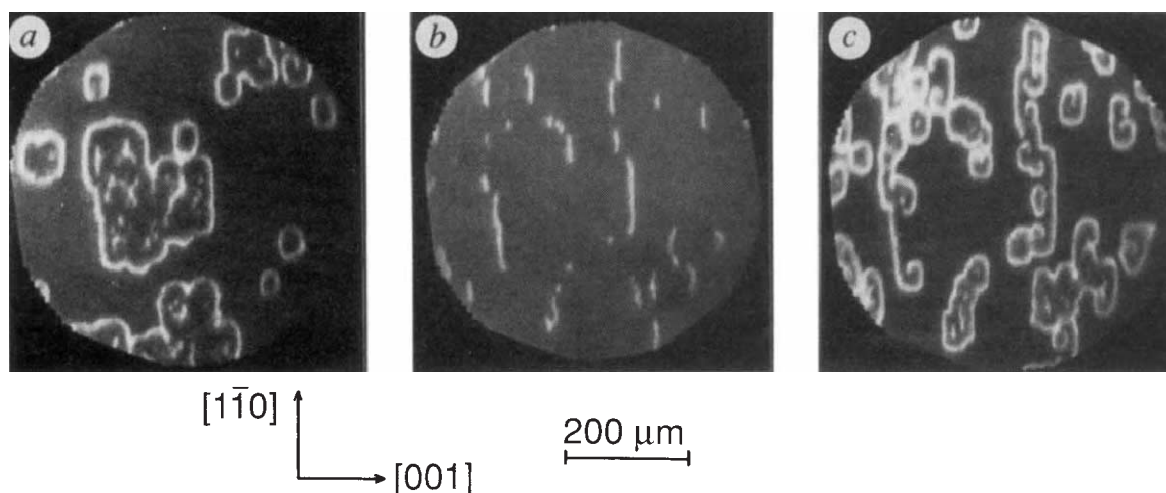


FIG. 3 Dependence of the excitability and anisotropy of front propagation on p_{H_2} . After the square-shaped target pattern shown in a had been created, p_{H_2} was reduced from 5×10^{-6} mbar to 4.5×10^{-6} mbar pro-

ducing the pattern shown in b. A subsequent increase of p_{H_2} to 5×10^{-6} mbar led to the formation of spirals (c). Experimental parameters; $T = 595$ K, $p_{\text{NO}} = 1.6 \times 10^{-6}$ mbar.

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Double-stranded inclusion complexes of cyclodextrin threaded on poly(ethylene glycol)

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MUCH attention has been focused recently on the design and construction of nanoscale structures by supramolecular assembly^{1–5}. One of the most promising approaches to constructing nanoscale structures is the use of specific interactions between polymers and receptors, as exemplified by biological systems. Recently, polyrotaxanes—complexes in which several cyclic molecules are threaded on the main chains^{6–11} or side chains¹² of polymers—have been synthesized with crown ethers or cyclodextrins as the cyclic components. Here we report the self-assembly of double-stranded inclusion complexes of poly(ethylene glycol) with γ -cyclodextrin, in which two polymer chains are threaded through the macrocycles. These complexes might be useful precursors to more complex supramolecular assemblies such as polycatenanes.

In the course of experiments on the preparation of inclusion complexes of cyclodextrins (CDs) with poly(ethylene glycol) (PEG), we found that γ -CD formed a trace amount of complexes with PEG. The amount of the complexes formed is so small that we have not yet been able to characterize them. But we found that some PEG derivatives, such as bis(3,5-dinitrobenzoyl)-poly(ethylene glycol) (PEG-DNB₂) and bis(2,4-dinitrophenylamino)-poly(ethylene glycol) (PEG-DNP₂), formed complexes with γ -CD to give crystalline compounds in high yields. On the other hand, α -CD did not form complexes with these PEG derivatives because the substituents at the end groups are too large to penetrate α -CD cavities. By using PEG with fluorescent probe groups attached to the ends, we have been able to establish that these complexes are composed of double chains of PEGs threaded through the γ -CDs.

PEG-DNB₂ (relative molecular mass, M_r , 1,500) was added to an aqueous solution of γ -CD at 2:1 (ethylene glycol unit: γ -CD) ratio, which is the α -CD:PEG complex ratio. We obtained a complex in 50% yield, that is, 50% of the γ -CD was complexed. In contrast, we found that all the PEG-DNB₂ had been complexed as shown by the disappearance of its characteristic yellow colour. We then added the same amount of PEG-DNB₂ again to the solution (twice as much as the α -CD:PEG complex ratio). This resulted in the complexation of a further 40% of the γ -CD, giving an overall total of 90% complexation.

We have isolated the complexes and measured their ¹H NMR spectra. By comparison of integrals of the peaks of CDs with that of PEG, four ethylene glycol units were found to be included in a single γ -CD cavity. In contrast, α -CD forms a single-chain complex with PEG in the ratio two ethylene glycol units to one CD regardless of the ratio in which the α -CD and PEG are mixed. γ -CD also forms complexes with poly(propylene glycol) (PPG) in 2:1 ratio¹³. These results suggest that γ -CD cavities include two chains of PEG.

To investigate further the nature of the complexes formed between cyclodextrins and PEG derivatives, we prepared complexes with the fluorescently labelled derivatives bis(1-naphthylacetyl)-PEG (PEG-1N₂) and bis(2-naphthylacetyl)-PEG (PEG-

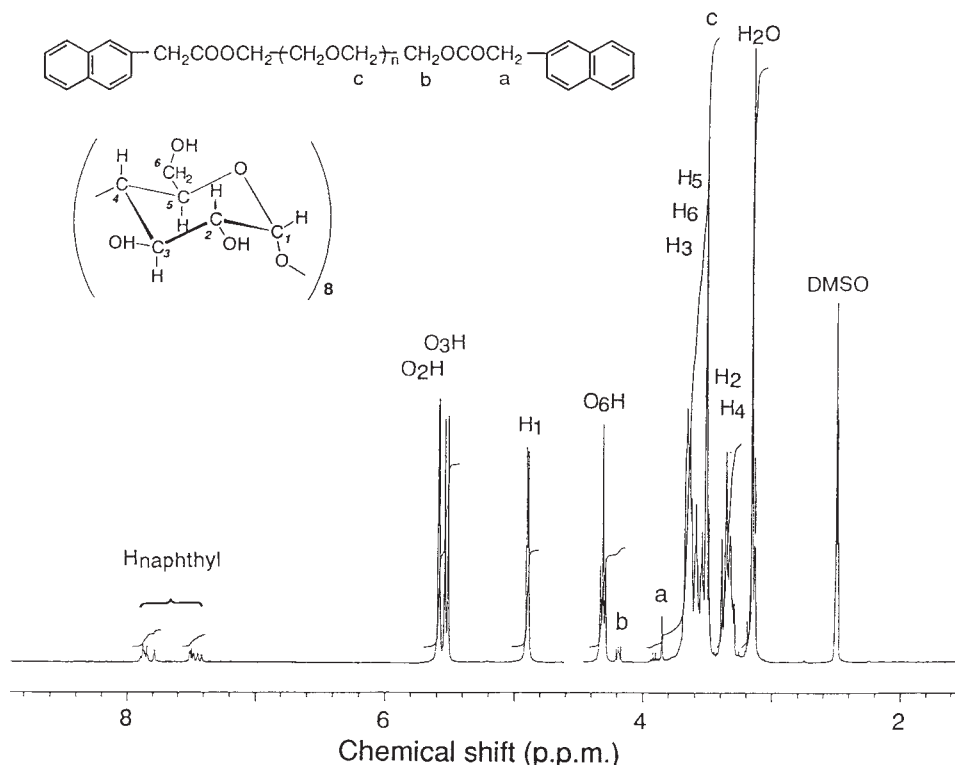


FIG. 1 270-MHz ¹H NMR spectrum of the complex between PEG-2N₂ and γ -CD in perdeuterated dimethyl sulphoxide (DMSO). Chemical shifts are referenced to dimethyl sulphoxide (δ 2.50).

TABLE 1 Complex formation between CD and PEG with various bulky end groups

	$R(\text{CH}_2\text{CH}_2\text{O})_n \text{CH}_2\text{CH}_2\text{R}'$			α -CD		γ -CD	
	R	R'	m_r^*	Yield (%)	Stoichiometry [†]	Yield (%)	Stoichiometry [‡]
PEG-DNB ₂			900	0	—	91	4:1
PEG-DNP ₂			1,300	0	—	85	4:1
			2,300	0	—	89	4:1
PEG-1N ₂			1,200	0	—	90	4:1
PEG-1N		-OCH ₃	900	76	2:1	77	4:1
PEG-2N ₂			1,300	56	2:1	89	4:1
PEG-2N		-OCH ₃	900	78	2:1	79	4:1

Abbreviations used; CD, cyclodextrin; PEG, poly(ethylene glycol); R, end group on PEG; M_r , relative molecular mass.

* The end groups contribute <10% of this relative molecular mass.

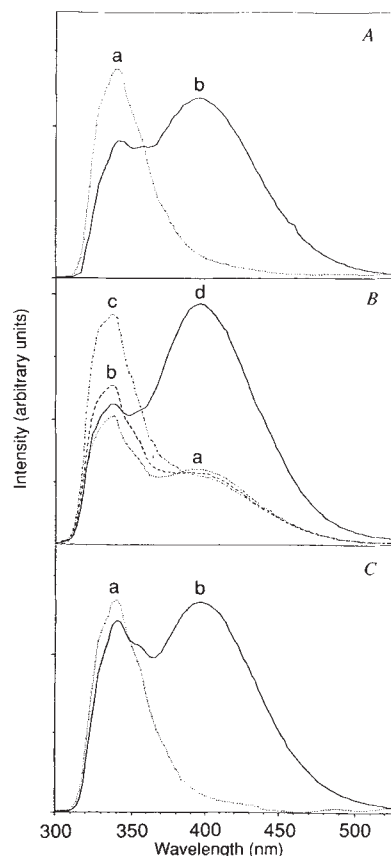
† The ratio between ethylene glycol units and CD as measured by ¹H NMR spectra, 2.0 ± 0.2 .

‡ The ratio between ethylene glycol units and CD as measured by ¹H NMR spectra, 4.0 ± 0.2 .

2N₂). We isolated the complexes of these polymers with γ -CD using standard methods¹³. NMR spectra indicate that again the complexes have a PEG: γ -CD ratio of 4:1 (Fig. 1). The emission spectra of the γ -CD:PEG-2N₂ complex show a large contribution from excimers, collective excitations arising from the interactions between two nearby naphthyl groups¹⁴, but only a small contribution from isolated ('monomeric') naphthyls (Fig. 2A, peak b). In contrast, the emission spectra of α -CD:PEG-2N₂ show only emission from isolated naphthyls (Fig. 2A, peak a). These results indicate that α -CD is threaded by just a single PEG chain whereas γ -CD is threaded by two. The spectra of PEG-1N₂ with CDs tell the same story (data not shown): in the absence of CDs, we see mainly monomer emission, with a small contribution from excimers; the addition of β - and α -CDs cause the monomer emission to increase and the excimer emission to decrease; but in the presence of γ -CD the emission is primarily from excimers.

These results show that two naphthyl groups can be included within the γ -CD cavity. There are two possible interpretations of this: an intermolecular complex (Fig. 3a) and intramolecular complex (Fig. 3b). To distinguish between these alternatives, we prepared monosubstituted naphthyl derivatives of PEG (PEG-2N). These gave similar spectra (Fig. 2C) to those with the disubstituted forms, indicating that the complexes must be double-stranded. Table 1 summarizes our results for complexation

FIG. 2 A, Emission spectra of the complexes of PEG-2N₂ with α -CD (trace a) and with γ -CD (b). The excitation wavelength is 280 nm. The bands at 340 and 400 nm are due to emission from the monomer and excimer respectively (see text). B, Emission spectra of PEG-2N₂ (7.6×10^{-6} M). Trace a, in the absence of CDs; trace b, in the presence of α -CD (1.5×10^{-2} M); trace c, in the presence of β -CD (1.5×10^{-2} M); and trace d, in the presence of γ -CD (1.5×10^{-2} M). C, Emission spectra of PEG-2N. Trace a, in the presence of α -CD; trace b, in the presence of γ -CD.



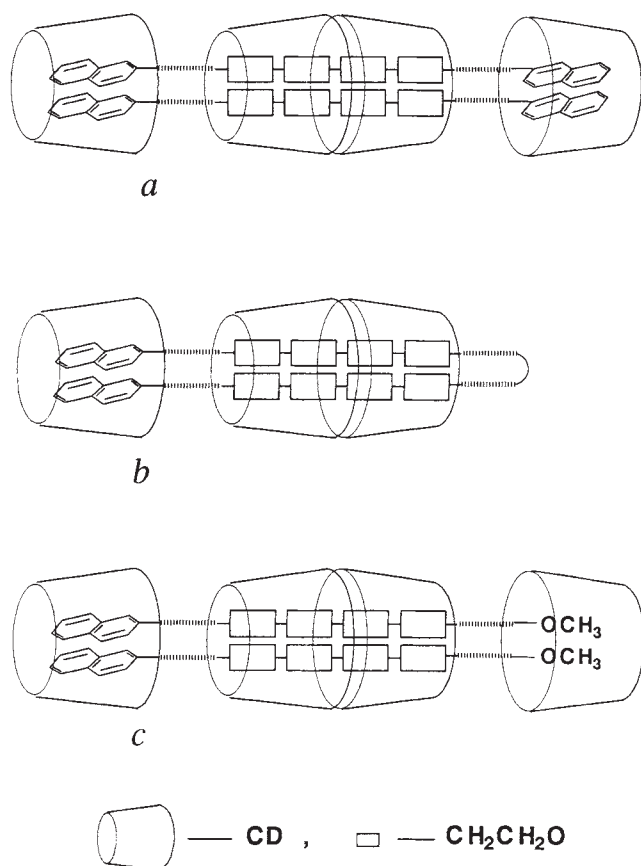


FIG. 3 Probable structures of complexes of mononaphthyl PEG: a, an intermolecular double-chain complex; b, an intramolecular single-chain complex; c, an intermolecular double-chain complex.

between γ -CD and a number of PEG derivatives with bulky end groups.

The diameter of γ -CD cavity is 8.5–9 Å which is twice as large as that of α -CD (4.5 Å). But the depth of the cavity of γ -CD is the same as those of α -CD and β -CD (7 Å), which corresponds to the length of two ethylene glycol units. Molecular model studies indicate that the γ -CD cavity is large enough to accommodate a double-chain of PEG, whereas the α -CD cavities are too small to do this.

This double-stranded inclusion phenomenon is reminiscent of the inclusion of the double helix of DNA by DNA polymerases. We anticipate that new kinds of supramolecular assembly might be prepared from these double-stranded complexes. Joining each pair of ends, for example, would produce catenanes in which 'beads' are threaded on double rings. □

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Stimulated anoxic biodegradation of aromatic hydrocarbons using Fe(III) ligands

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CONTAMINATION of ground waters with water-soluble aromatic hydrocarbons, common components of petroleum pollution, often produces anoxic conditions under which microbial degradation of the aromatics is slow^{1–7}. Oxygen is often added to contaminated ground water to stimulate biodegradation, but this can be technically difficult and expensive^{1,5–8}. Insoluble Fe(III) oxides, which are generally abundant in shallow aquifers, are alternative potential oxidants, but are difficult for microorganisms to access⁹. Here we report that adding organic ligands that bind to Fe(III) dramatically increases its bioavailability, and that in the presence of these ligands, rates of degradation of aromatic hydrocarbons in anoxic aquifer sediments are comparable to those in oxic sediments. We find that even benzene, which is notoriously refractory in the absence of oxygen, can be rapidly degraded. Our results suggest that increasing the bioavailability of Fe(III) by adding suitable ligands provides a potential alternative to oxygen addition for the bioremediation of petroleum-contaminated aquifers.

Microorganisms can degrade water-soluble aromatic hydrocarbons under anoxic conditions^{10–14}. But the rates of anoxic degradation are much slower than under oxic conditions and aromatic hydrocarbons, especially the highly toxic benzene, tend to persist in anoxic ground water^{1–7}. This is generally attributed to the fact that, in addition to serving as an electron acceptor for microbial respiration, O₂ is also a direct reactant in oxic hydrocarbon oxidation^{7,15}. The addition of nitrate (NO₃[–]) to anoxic ground water has been considered as a potential alternative bioremediation strategy to O₂ additions. Nitrate can serve as an electron acceptor for the anoxic oxidation of toluene and xylenes, but most recent studies have indicated that benzene is not degraded with nitrate as the electron acceptor under anoxic conditions^{11,16–20}.

Before contamination, insoluble Fe(III) oxides are generally the most abundant potential electron acceptors for oxidation of organic matter in shallow aquifers⁹. Geochemical studies have suggested that microbial Fe(III) reduction is an important process for the anoxic oxidation of some organic contaminants in polluted aquifers^{21–23}, but Fe(III) reduction is much slower than aerobic respiration in removing aromatic hydrocarbons. Although this could possibly be due to slow metabolism of the aromatic ring in the absence of O₂, we hypothesized that the poor ability of Fe(III)-reducers to access highly insoluble Fe(III) oxides might be a factor limiting the rate of aromatic hydrocarbon degradation.

Organic ligands that bind to Fe(III) oxides may make the Fe(III) more available for microbial reduction. For example, with lactate (a compound that is easily metabolized under anaerobic conditions) serving as the electron donor, the binding of nitrilotriacetic acid (NTA, C₆H₅NO₆) onto the Fe(III) oxide (goethite) permitted *Shewanella putrefaciens* (formerly *Pseudomonas* strain 200), to reduce the Fe(III) much faster²⁴.

In order to determine if Fe(III) ligands might stimulate aromatic hydrocarbon degradation in anoxic aquifers, sediments and ground water were collected from a shallow water-table

aquifer at the Defense Fuel Supply Center in Hanahan, South Carolina. The hydrology and chemistry of this petroleum-contaminated aquifer has recently been described in detail²⁵. Aromatic hydrocarbons (primarily benzene and toluene) are the principal contaminants of concern in the ground water. Like most petroleum-contaminated aquifers, the aquifer at Hanahan contains extensive regions in which microbial metabolism has depleted O₂ and nitrate from the ground water, but Fe(III) oxides are present which could potentially serve as an electron acceptor for microbial oxidation of the hydrocarbon contaminants. Sediments were collected from such a site, known as MW 20. Fe(III) reduction was identified as the predominant terminal electron accepting process at this site by field measurements of dissolved H₂ in the ground water and by studies on microbial metabolism in laboratory incubations of aquifer material²⁶.

Additions of NTA greatly stimulated the degradation of toluene in MW 20 sediments (Fig. 1a). In sediments in which the microorganisms had been killed with heat before the incubation, there was a slow loss of toluene over time, presumably due to absorption of toluene into the butyl rubber stoppers¹⁴. Addition of NTA to the heat-sterilized sediments had no effect on the rate of the abiotic toluene loss. Rates of toluene uptake were somewhat faster in live sediments than in the heat-sterilized sediments, suggesting Fe(III) reducers were naturally slowly degrading toluene. In contrast, with the addition of NTA, toluene rapidly disappeared after a brief lag. When toluene was repeatedly added back, it was again quickly removed. When [ring-¹⁴C]-toluene was added to NTA-treated, toluene-adapted sediments, 80 ± 1% (mean ± standard deviation, *n* = 3) of the added radioactivity was recovered as ¹⁴CO₂, indicating that toluene was being oxidized to carbon dioxide. The complete removal of the added toluene within a day in adapted NRA-treated sediments (Fig. 1a) compares favourably with a first-order rate constant of 1.8 d⁻¹ for toluene oxidation in oxic sediments from the Hanahan aquifer, and is more than 50 times faster than the rates of toluene degradation in nitrate-treated, anoxic Hanahan sediments (P. M. Bradley and F.H.C., unpublished observations).

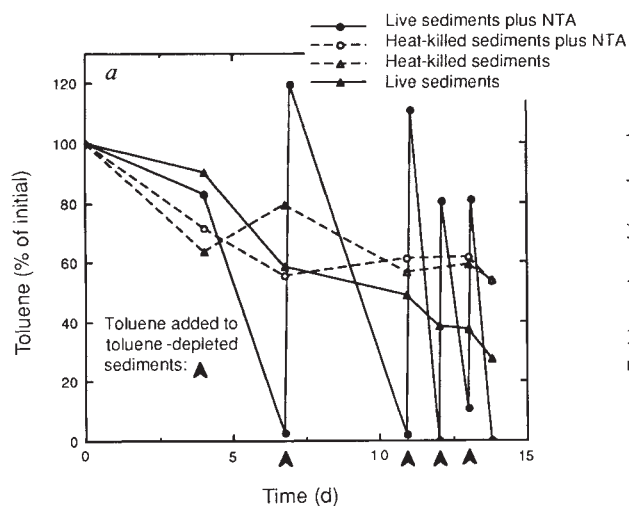


FIG. 1 Stimulation of toluene degradation (a) and Fe(III) reduction (b) by nitrilotriacetic acid (NTA) in Fe(III) oxide-containing sediments under anoxic conditions.

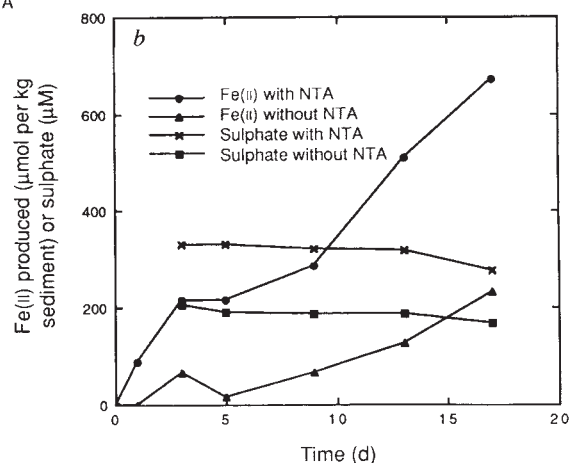
METHODS. a, Sediments and associated ground water (~30 ml) were incubated at the approximate *in situ* temperature of 20 °C under N₂/CO₂ (93:7) in serum bottles (36 ml) sealed with thick butyl rubber stoppers. The bottles were incubated upside down to minimize contact between toluene in the headspace and the stoppers. Heat-sterilized sediments were prepared by autoclaving (121 °C, 1 h) on three consecutive days before incubation. Toluene was added at zero time and where indicated (arrowheads) from an anoxic stock of toluene dissolved in water (0.1 ml) in order to provide ~10 µmol of toluene per kg of

TABLE 1 Fe(III) reduction during oxidation of benzene to carbon dioxide

Bottle	Fe(II) produced (µmol)	Benzene oxidized (µmol)	Fe(II) expected (µmol)	Fe(II) observed/expected
1	16.9	0.548	16.4	1.03
2	17.7	0.608	18.2	0.97
3	24.7	0.620	18.6	1.33

Benzene-adapted NTA-treated sediments (~16 g per bottle) were treated with poorly crystalline Fe(III) oxide (2% vol/vol) to ensure that metabolism did not become Fe(III) limited, and were incubated as in Fig. 2 until benzene was depleted. HCl-extractable Fe(II) concentrations²⁷ were determined, and benzene was then added to the sediments as in Fig. 2, with the exception that ¹⁴C-benzene was included. Controls received equal additions of water without benzene. After seven additions of benzene, the moles of benzene oxidized were calculated from the amount of ¹⁴CO₂ produced and the specific activity of the benzene. Fe(II) produced was determined as the amount of Fe(II) accumulation in benzene-treated sediments minus Fe(II) produced in the controls without benzene. The amount of Fe(II) expected to be produced was calculated based on the stoichiometry of 30 mol of Fe(III) reduced per mol of benzene oxidized to carbon dioxide.

In order to determine whether the faster rates of toluene degradation in the NTA-treated sediments could be attributed to NTA stimulating Fe(III) reduction, Fe(II) and sulphate concentrations were monitored over time in MW 20 sediments with toluene added (Fig. 1b). Rates of Fe(II) production from Fe(III) reduction were markedly faster when NTA was added to the sediments. There was no significant depletion of sulphate, suggesting that toluene degradation was coupled only to Fe(III) reduction. NTA stimulation of aromatic hydrocarbon degradation could not be attributed to NTA providing either a source of organic carbon or fixed nitrogen for two reasons. (1) Subsequent studies indicated there was no loss of NTA over time in similar incubations, and (2) addition of a readily degradable organic



sediment. NTA was added from a concentrated anoxic stock (1 M) in order to provide ~2 mmol of NTA per kg of sediment. Toluene was measured by sampling the headspace with a syringe and needle and analysing with gas chromatography. Incubations were the same for b as in a, with the exception that the sediment volume was 100 ml and the bottle volume was 160 ml. HCl-extractable Fe(II) and dissolved sulphate were measured as previously described²⁷. Toluene was added after bottles were opened for Fe(II) determinations in order to maintain toluene concentrations at ~10 µM. Except where noted, all values in this and subsequent figures are the means of triplicate incubations. The standard error in the means for aromatic hydrocarbon and Fe(II) determinations were 5% and 11%, respectively.

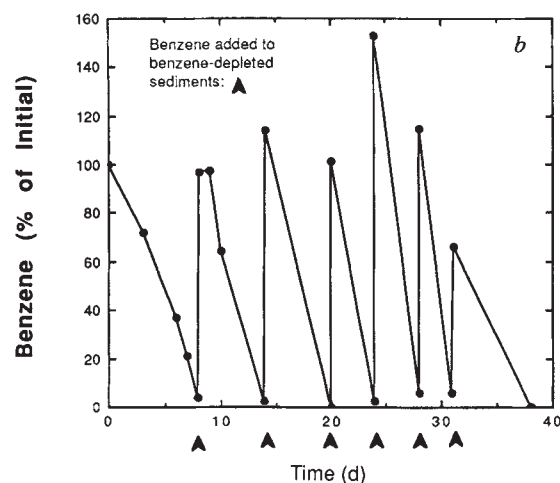
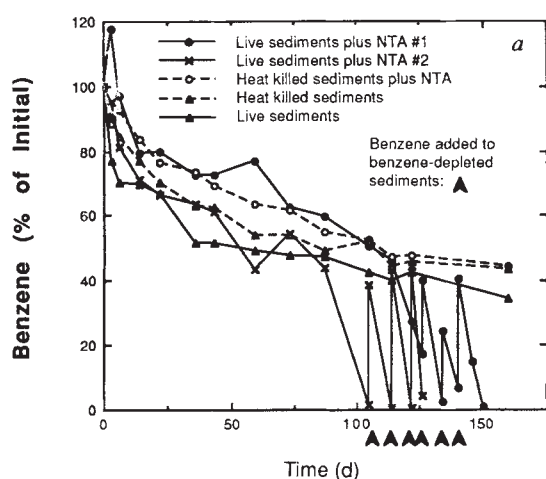


FIG. 2 Loss of benzene over time in Fe(III) oxide-containing sediments under anoxic conditions. In the presence of NTA, benzene was rapidly degraded. The long lag in adaption to benzene degradation in the presence of NTA (a) was prevented when, at the time of NTA additions, the sediments were inoculated with microorganisms from a benzene-adapted sediment (b). The lag periods in adaption to benzene in the sediments (a) varied, and thus the data for these sediments are shown separately rather than showing the mean of the three samples. For

clarity, the data for sediment no. 3 (which was similar to sediment no. 2) is not shown.

METHODS. Incubations and analyses were the same as in Fig. 1, with the exception that benzene was added to the sediments instead of toluene. At 126 days of incubation (a) sediments from sediment no. 2 were used to inoculate fresh sediments that were then amended with NTA (b).

substrate (4 mM acetate) or a source of fixed nitrogen (1 mM ammonium chloride), or both, did not stimulate toluene degradation.

NTA also stimulated benzene degradation (Fig. 2). Initially, the loss in NTA-treated sediments was comparable to that in controls. However, after lag periods of 87–122 days, benzene disappeared rapidly from the three live sediments (Fig. 2a). Once

benzene was depleted, it was added back to the sediments and was removed without a lag. In contrast, there was little or no loss of benzene from the other treatments during the same period. Coupled with the fact that in studies with numerous Hanahan sediment samples, benzene degradation was only observed in the presence of NTA, this demonstrates that the benzene degradation could not be attributed to traces of O_2 leaking through a faulty stopper.

When fresh aquifer sediments were inoculated with some of the sediments that had been adapted for benzene degradation, and NTA was added at the same time, the benzene was rapidly degraded without a lag (Fig. 2b). The rates of benzene loss in the benzene-adapted sediments (Fig. 2a and 2b) were comparable to those that are typically observed in incubations of oxic aquifer material¹.

The enhanced benzene loss from the live NTA-treated sediments could be attributed to benzene oxidation to carbon dioxide because when ^{14}C -benzene was added to such sediments, $86 \pm 2\%$ (mean $\pm$ standard deviation, $n = 3$) of the added radioactivity was recovered as $^{14}CO_2$. Measurements of benzene oxidation and Fe(III) reduction in benzene-adapted sediments (Table 1) demonstrated that the increased Fe(III) reduction in the presence of benzene was consistent with complete oxidation of benzene to carbon dioxide, with Fe(III) serving as the sole electron acceptor: $C_6H_6 + 30 Fe(III) + 12 H_2O \rightarrow 6 CO_2 + 30 Fe(II) + 30 H^+$. It seems likely that there are Fe(III) reducers that grow by benzene oxidation. This is because sediment-free enrichments, with benzene as the sole electron donor and Fe(III)-NTA as the electron acceptor, have been established by consecutive transfer of the sediment inoculum in a medium containing ground water from the site, synthetic poorly crystalline Fe(III) oxide and NTA. However, attempts to isolate a pure culture of a benzene-oxidizing Fe(III) reducer have as yet been unsuccessful.

Further evidence that NTA stimulated aromatic hydrocarbon degradation by stimulating Fe(III) reduction was provided by the following: NTA additions did not stimulate aromatic hydrocarbon degradation in aquifer material which was depleted of Fe(III) oxides and in which sulphate reduction or methane production was the terminal electron accepting process (data not shown). However, when Fe(III) chelated with NTA was added to sediments in which methanogenesis was the terminal electron-

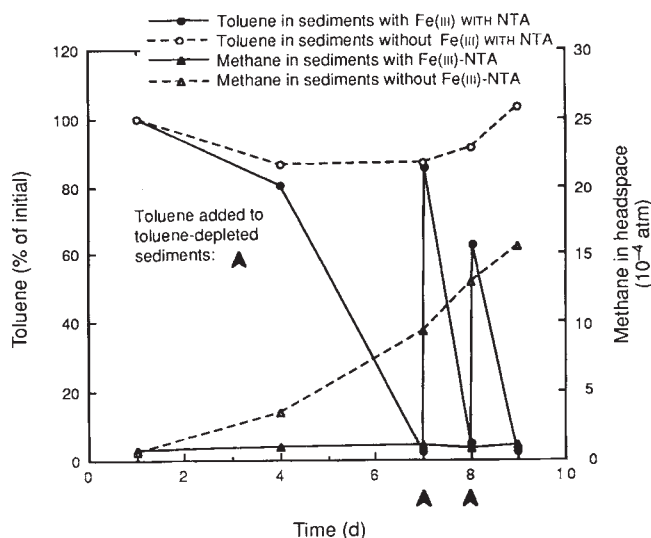


FIG. 3 Toluene and methane concentrations over time in aquifer material in which methane production was initially the terminal electron accepting process. Toluene persisted in the methanogenic sediments but was rapidly degraded when Fe(III)-NTA was added. The degradation of toluene was associated with an inhibition of methane production, due to a switch in electron flow to Fe(III) reduction in the presence of Fe(III).

METHODS. Incubations and analyses were the same as in Fig. 1, with the exception that the sediments were from site MW1, a methanogenic portion of the aquifer. An anoxic stock of Fe(III)-NTA was prepared as previously described²⁸ and added to give ~ 2 mmol per kg of sediment.

accepting process, aromatic hydrocarbon degradation was stimulated (Fig. 3 and other data, not shown).

These results demonstrate that the slow degradation of aromatic hydrocarbons in contaminated aquifers is not due solely to a lack of O_2 . Aromatic hydrocarbons can be rapidly degraded in the absence of O_2 when either Fe(III) oxides are made more available for microbial reduction or chelated Fe(III) is added. Although NTA was used in the studies reported here, subsequent studies have indicated that ethylenediaminetetraacetic acid (EDTA), another Fe(III) ligand, stimulates aromatic hydrocarbon degradation at least as well as NTA. These findings suggest that the addition of an Fe(III) ligand (when Fe(III) oxides are present in the aquifer) or soluble, chelated Fe(III) (when Fe(III) oxides have been depleted) is a potential strategy for the bioremediation of petroleum-contaminated aquifers. □

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Absence of an aluminous phase in the upper part of the Earth's lower mantle

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RECENT high-pressure experiments^{1–3} suggest that the Earth's lower mantle is composed of $MgSiO_3$ and $CaSiO_3$ perovskites, $(Mg, Fe)O$ magnesiowüstite, and a minor but significant amount of aluminous phases, such as majorite garnet or an unidentified Al-rich phase. The stability of majorite garnet and the nature of any such aluminous phase, however, are controversial issues relevant to the mineralogy of the lower mantle^{4–7}. Here I report an experimental study of the phase transformations that occur in a pyrolite mantle composition with increasing pressure from 23 to 28 GPa (equivalent to ~650–770 km depth in the mantle). The results demonstrate that majorite garnet completely transforms to perovskite structures at pressures above 26 GPa (>720 km depth). Al_2O_3 is accommodated mainly in $MgSiO_3$ perovskite, and no separate aluminous phase was observed at higher pressures, leading to the conclusion that the upper part of the Earth's lower mantle is composed only of two perovskites and magnesiowüstite.

Our high-pressure experiments were performed in a multianvil 6–8 type apparatus. Details of the experimental technique have been reported elsewhere^{8,9}. Pyrolite¹⁰ was chosen as a representative mantle composition. A starting material of this composition was prepared by intimately mixing oxide and carbonate compounds, forming a pellet, and firing for 24 h at 1,000 °C. Very fine powders of SiO_2 and Al_2O_3 (grain size <0.02 µm) were used to facilitate chemical equilibration during the run. Electron microprobe analyses of the sintered starting material yielded a composition (wt%) as follows: SiO_2 , 45.2; TiO_2 , 0.3; Al_2O_3 , 3.9; Cr_2O_3 , 0.5; FeO , 8.1; MgO , 37.5; NiO , 0.3; CaO , 3.8; and Na_2O , 0.3. The starting material was enclosed in a platinum capsule and subjected to pressures of 23–28 GPa, at temperatures of 1,500–1,600 °C for typically 20 min. The uncertainties in pressure and temperature were within 1 GPa and 50 °C,

respectively⁸. After quenching, the samples were characterized by optical microscopy, X-ray powder diffraction with a 57.3 mm Debye Scherrer camera, and electron microprobe analyses with both wavelength dispersive and energy dispersive systems.

An assemblage of γ -(Mg, Fe) $_2SiO_4$ + $CaSiO_3$ -rich perovskite (the latter converted to an amorphous phase on release of pressure) + majorite garnet was formed in a run at 23 GPa and 1500 °C. The γ -phase and majorite crystallized as grains of typically 2–10 µm in dimension, whereas $CaSiO_3$ -rich perovskite exhibited a peculiar aggregate texture (Fig. 1a). The chemical compositions of these phases are listed in Table 1. The phase assemblage and the phase chemistry of this run are consistent with earlier results using a hydrous silicate starting material, of ‘pyrolite minus olivine’ composition³, and natural peridotites^{2,11}. Moreover, I have confirmed that all coexisting phases are fairly uniform in composition throughout the charge, except for minor heterogeneity in magnesiowüstite (normally within 2–3% in terms of $Mg/(Mg + Fe)$); no obvious chemical zoning was observed in any of these phases. These observations suggest that chemical equilibrium was attained in the experiments reported here.

The pyrolite composition transformed into an assemblage of γ -(Mg, Fe) $_2SiO_4$ + $MgSiO_3$ - and $CaSiO_3$ -rich perovskites (abbreviated as Mg-pv and Ca-pv hereafter) + magnesiowüstite + majorite garnet at 24 GPa, and the γ -phase completely disappeared at 25 GPa. These orthorhombic and cubic perovskites had chemical compositions close to their respective end-members, and both of these perovskites contained appreciable amounts of Al_2O_3 (Table 1). The magnesiowüstite was enriched in FeO as compared to Mg-pv, in accordance with the Mg–Fe partitioning reported in the Mg_2SiO_4 – Fe_2SiO_4 system¹². This magnesiowüstite also contained up to ~1 wt% of NiO, which is consistent with recent experimental results that suggest some of the magnesiowüstite inclusions in diamond may have originated from the lower mantle¹³.

Only faint X-ray diffraction peaks were observed for majorite garnet at 25 GPa, and no such peaks were identified at 26 GPa. It was also extremely difficult to find the garnet crystals in this run under the electron microscope, and only a few reliable analyses were obtained for majorite garnet at this pressure. The electron microprobe analyses demonstrated that the composition of majorite garnet approaches that of ‘normal’ stoichiometric gar-

TABLE 1 Chemical compositions of the coexisting phases in selected runs

	E293 (23 GPa, 1,500 °C)			E291 (24 GPa, 1,500 °C)					E296 (25 GPa, 1,500 °C)				E631 (28 GPa, 1,600 °C)		
	γ	Ca-pv	gt	γ	Mg-pv	mw	Ca-pv	gt	Mg-pv	mw	Ca-pv	gt	Mg-pv	mw	Ca-pv
SiO ₂	41.48	45.65	51.64	42.43	53.42	0.32	48.42	49.79	53.18	0.22	45.72	47.62	53.07	1.01	49.06
TiO ₂	—	4.04	0.20	—	0.36	—	0.56	0.18	0.26	—	0.50	0.10	0.31	—	0.14
Al ₂ O ₃	—	1.25	10.31	—	3.68	0.20	1.65	12.34	3.91	0.15	1.97	13.86	4.92	0.97	1.15
Cr ₂ O ₃	—	—	1.32	—	0.52	0.32	0.20	1.07	0.36	0.32	0.15	1.10	0.56	0.98	—
FeO	8.62	0.61	3.98	9.29	6.34	22.26	0.48	4.00	6.69	21.69	1.19	4.56	7.04	20.47	0.48
MgO	48.71	1.52	27.57	47.66	34.27	73.93	1.69	22.15	34.45	75.59	2.28	24.60	33.43	72.56	1.19
NiO	0.45	—	—	0.30	—	0.68	—	—	—	0.80	—	0.32	0.15	1.12	0.20
CaO	—	41.45	4.39	—	0.20	—	40.73	8.59	0.30	—	38.24	4.16	0.25	—	43.10
Na ₂ O	—	—	0.51	—	—	0.50	0.20	1.21	—	0.28	0.20	1.06	0.23	1.22	0.21
Total	99.26	94.52	99.92	99.68	98.79	98.21	93.93	99.33	99.15	99.05	90.25	97.38	99.96	98.33	95.53
No. of oxygens	4	3	12	4	3	1	3	12	3	1	3	12	3	1	3
Si	1.017	0.933	3.580	1.035	0.937	0.002	0.984	3.521	0.931	0.002	0.970	3.411	0.924	0.008	0.987
Ti	—	0.062	0.010	—	0.005	—	0.009	0.010	0.003	—	0.008	0.005	0.004	—	0.002
Al	—	0.030	0.848	—	0.076	0.002	0.040	1.029	0.081	0.001	0.049	1.170	0.101	0.009	0.027
Cr	—	—	0.072	—	0.007	0.002	0.003	0.060	0.005	0.002	0.003	0.062	0.008	0.006	—
Fe	0.177	0.010	0.231	0.190	0.093	0.142	0.008	0.237	0.098	0.137	0.021	0.273	0.103	0.129	0.008
Mg	1.781	0.046	2.849	1.734	0.896	0.840	0.051	2.336	0.899	0.848	0.072	2.628	0.868	0.818	0.036
Ni	0.009	—	—	0.006	—	0.004	—	—	—	0.005	—	0.018	0.002	0.007	0.003
Ca	—	0.908	0.325	—	0.004	—	0.887	0.651	0.006	—	0.869	0.319	0.005	—	0.929
Na	—	—	0.068	—	—	0.007	0.008	0.166	—	0.004	0.008	0.147	0.008	0.018	0.008
Sum	2.983	1.990	7.983	2.965	2.017	0.999	1.990	8.008	2.023	0.999	2.000	8.033	2.022	0.994	2.001
Mg*	91.0		92.5	90.1	90.6	85.6		90.8	90.2	86.1		90.6	89.4	86.3	

Chemical compositions given in wt%. Symbols used: γ , γ -(Mg,Fe)₂SiO₄; Ca-pv, CaSiO₃-rich perovskite; gt, majorite garnet; Mg-pv, MgSiO₃-rich perovskite; mw, magnesio-wüstite; Mg* = 100 × Mg/(Mg + Fe); —, under the detection limit (<0.1 wt%). The oxide totals for Ca-pv are substantially lower than 100% because of the formation of aggregates of minute amorphous grains on quenching (see Fig. 1). The chemical composition data for runs E289 (26 GPa, 1,500 °C) and E628 (27 GPa, 1,500 °C) were omitted from the table because they display essentially the same phase assemblages as that of E631. The compositions of the coexisting phases also remained virtually the same in these runs, except for some increase in the abundances of relatively minor elements, such as Al₂O₃, Cr₂O₃, NiO and Na₂O in magnesio-wüstite with increasing pressure.

net (that is, the Al₂O₃ content increases) with increasing pressure between 23 and 26 GPa.

At pressures of 27 and 28 GPa, majorite garnet completely disappeared and only three phases, Mg-pv, Ca-pv and magnesio-wüstite, were confirmed from both X-ray diffraction and electron microscope measurements (Table 1, Fig. 1b). The lattice parameters of coexisting magnesio-wüstite and Mg-pv synthesized at 28 GPa (run E631 in Table 1) were measured by an X-ray diffractometer utilizing an X-ray source with a rotating copper anode. The lattice parameter of magnesio-wüstite was $a=4.226$ (1) Å, which agrees well with that estimated from its composition ($a=4.225$ Å), assuming that the lattice parameter changes linearly with the Fe/Mg ratio. The coexisting Mg-pv had the lattice parameters $a=4.788$ (1), $b=4.945$ (2) and $c=6.929$ (2) Å, which are significantly larger than those of pure Mg-pv ($a=4.775$, $b=4.929$ and $c=6.897$ Å)¹⁴. These values are consistent with those estimated from the chemical composition of the present Mg-pv, based on the composition–lattice parameter relationships^{15,16}. Mass-balance calculations also suggest no additional phases present at more than 0.5 wt% are required to account for the composition of the Al₂O₃ inventory of pyrolite. Mg-pv accepts most of the Al₂O₃, while some is incorporated into the Ca-pv structure.

The present study is pertinent to earlier work by Takahashi and Ito², who studied the phase transformations in a natural peridotite sample (PHN-1611) at pressures up to 26 GPa and temperatures of 1,500–1,650 °C, in a multianvil apparatus. The chemical composition of this peridotite is close to that of the present pyrolite, and similar experimental results were obtained on the gross nature of the phase transformations. However, significant discrepancies exist between these two studies.

Takahashi and Ito² reported that Ca-pv has a composition similar to diopside (Ca_{0.5}Mg_{0.5})SiO₃ rather than CaSiO₃, as observed in the present study. This discrepancy is probably due to metastable growth of a CaMg perovskite phase from grains of former diopside composition in Takahashi and Ito's run. In fact, these authors note the presence of pseudomorphs formed from original crystals of enstatite and diopside pyroxenes (that

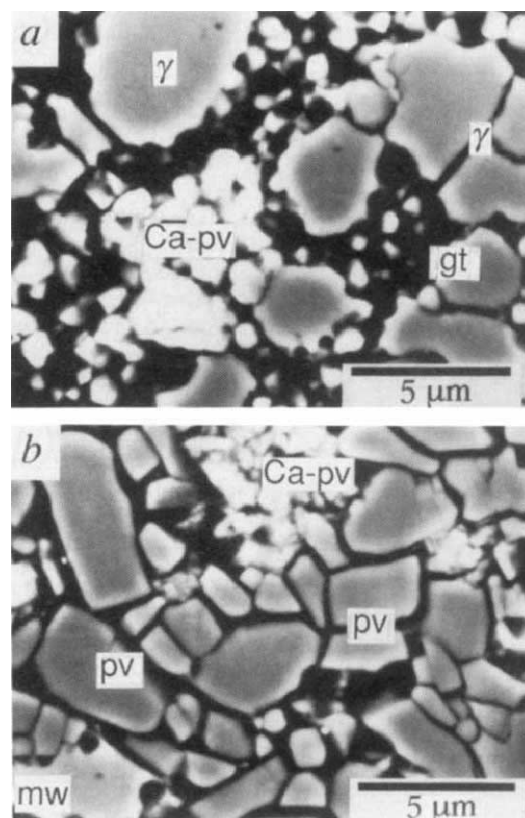


FIG. 1 Back-scattered electron images of the run products at 23 GPa (a) and 27 GPa (b). a, Equal-sized grains of γ -(Mg, Fe)₂SiO₄ (denoted by ' γ ' on Figure) and majorite garnet (gt) coexist with brighter CaSiO₃ perovskite, now converted to aggregates of an amorphous material (Ca-pv). b, MgSiO₃ perovskite (pv) often crystallizes in rectangular shapes, while magnesio-wüstite (mw) usually forms irregular or rounded crystals. The interstitial bright aggregates are amorphized former CaSiO₃ perovskite.

were present in their natural peridotite starting material) in some of the run products. More recent experimental studies^{17–19} have established that diopside actually decomposes into assemblages of Ca-pv plus other magnesian phases at pressures greater than 17–18 GPa, in accordance with the present results.

Takahashi and Ito reported that an unknown Al-rich phase was present at pressures of 24 and 26 GPa, at a temperature of 1,600 °C, although virtually no data have been reported on the chemical composition and the crystallographic structure of this phase, except for a few X-ray powder diffraction peaks. A number of experimental studies have subsequently been made to identify this Al-rich phase using simple compounds as starting materials. As a result, several new aluminous high-pressure phases have been reported^{4–7}, which may be candidates for the Al-rich phase in the lower mantle. However, the present results on a more realistic mantle composition suggest that none of these aluminous phases would exist in the lower mantle, and that most of the alumina would reside in Mg-pv. This conclusion is in accord with the result that orthorhombic perovskite in the $\text{MgSiO}_3\text{--Mg}_3\text{Al}_2\text{Si}_3\text{O}_{12}$ system can accommodate up to 25 wt% of Al_2O_3 at pressures greater than 25–30 GPa (refs 8,16,20).

The apparent conflict regarding the inventory of Al_2O_3 between the present study and Takahashi and Ito² is probably due to metastable growth of Mg-pv in the latter study. Takahashi and Ito² reported that Mg-pv contains only 1.2 wt% Al_2O_3 at 26 GPa. This alumina content is essentially the same as that of the enstatite (1.3 wt%) in their rock starting material, suggesting that the composition of this Mg-pv reflects that of the starting enstatite, as is the case for Ca-pv.

Absence of any Al-rich phase in the upper part of the lower mantle (except for the very top region between 660 and 720 km, where certain amounts of majorite garnet may exist) is consistent with an earlier study on a natural peridotite sample²¹, the composition of which is close to that of pyrolite. Only two perovskites and magnesiowüstite were confirmed by *in situ* X-ray measurements of the sample under pressure in a diamond anvil-cell at 54 GPa (~1,350 km depth), after heating at a peak temperature of 2,200 (± 200) K. It is therefore likely that no separate aluminous phases are present throughout most regions in the upper half of the lower mantle.

Figure 2 shows the changes of mineral proportions in the pyrolite composition as a function of pressure, based on mass-balance calculations using the present experimental data. It is clear that majorite garnet almost completely disappears at pressures greater than 26 GPa, equivalent to a depth of 720 km in the lower mantle. The calculated mineral proportions at these pressures are 76–77 vol% (Mg-pv), 15–16 vol% (magnesiowüstite) and ~8 vol% (Ca-pv). These results are virtually the same as the proportions estimated at 54 GPa (77, 16 and 7 vol%, respectively) in the peridotite composition²¹. Accordingly, no significant changes in phase proportions would be expected in pyrolite or peridotite compositions at depths corresponding to 26–54 GPa (720–1,350 km depth).

Some of the representative seismic velocity profiles^{22,23} suggest the presence of steep gradients at depths down to ~800 km in the lower mantle. This has been attributed to possible minor phase transformations in the mantle composition^{1–3}. However, the present results demonstrate that no phase transformations occur in representative mantle compositions at depths greater than 720 km. Thus the steep gradients in the uppermost lower mantle, if confirmed, must be due to other causes, such as the presence of chemical or thermal heterogeneities in this depth region.

It has been proposed that the lower mantle may have a different chemical composition compared to that of the upper mantle^{24–26}. Model lower-mantle compositions encompassing chondrite to pyroxenite are characterized by higher Si/Mg ratios than those of pyrolite or peridotite mantle models, whereas the Al_2O_3 contents of these compositions do not differ significantly. Chondrite-pyroxenite compositions would crystallize into

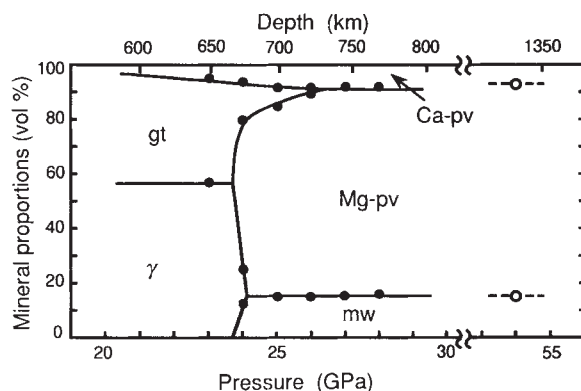


FIG. 2 Mineral proportions in a pyrolite composition as a function of pressure or depth. Filled circles represent the results of mass-balance calculations using the present experimental data, while the open circles represent the results of experiments on a natural peridotite sample using a diamond-anvil cell²¹. Symbols used: γ , γ -(Mg, Fe) $_2$ SiO $_4$, gt, majorite garnet; Ca-pv, CaSiO $_3$ -rich cubic perovskite; Mg-pv, MgSiO $_3$ -rich orthorhombic perovskite; mw, (Mg, Fe)O magnesiowüstite.

assemblages with higher perovskite/magnesiowüstite ratios than those in pyrolite-peridotite. Because perovskites accept substantial amounts of aluminium in their crystal structures, it is even more unlikely that separate aluminous phases would be stabilized in any hypothetical chondrite/pyroxenite lower mantle.

Basaltic compositions (which have a higher aluminium content than the model mantle composition studied here) have been found to crystallize into an assemblage with a new aluminous phase at pressures above 25 GPa (ref. 9). It has also been found that majorite garnet persists to pressures of at least 28 GPa in basaltic compositions⁹, which is in contrast to the stability of majorite in pyrolite. The complete transformation of majorite into dense perovskite structures by ~26 GPa in the pyrolite composition confirms our earlier conclusion that the subducted basaltic crust would become substantially less dense than pyrolite in a certain depth interval (650–800 km) within the lower mantle.⁹ □

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Primitive fossil rodent from Inner Mongolia and its implications for mammalian phylogeny

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THE evolutionary origin of rodents is obscured by the group's sudden and highly transformed first appearance in the fossil record^{1,2} in the latest Paleocene. We report here the discovery of nearly complete dental remains of an extraordinary new primitive rodent from strata of transitional Paleocene–Eocene age in Inner Mongolia, China. The strikingly conservative morphological features of this taxon, *Tribosphenomys minutus*, gen. et sp. nov., substantially modify previous ideas about the ancestral rodent morphotype, which in turn has important implications for understanding the origin of rodents and their relationship to other eutherian mammals. This new fossil, in conjunction with recent morphological³ and molecular^{4,5} evidence confirming rodent monophyly, indicates the need for a reassessment of phylogenetic affinities among gliriform eutherians. Our results indicate a sister-group position of the new taxon to other rodents, and support the alliance of lagomorphs (rabbits) and rodents (cohort Glires). They also suggest the paraphyly of an extinct assemblage, the 'Eurymylidae', and reveal an unexpectedly complex pattern of character evolution near the ancestry of Rodentia.

Cohort Glires Linnaeus, 1758

Order Rodentia Bowdich, 1821

Superfamily incertae sedis,

Family ?Alagomyidae Dashzeveg, 1990

Tribosphenomys minutus gen. et sp. nov.

Etymology. *Tribosphen*, Greek, meaning grind and wedge, in reference to its primitive, triangular crown pattern; *mys*, Greek, mouse; and *minutus*, Latin, in reference to its small size.

Holotype. V10775 (Institute of Vertebrate Paleontology and Paleoanthropology, Beijing, China), collected by J.M. and A.W. in 1993. The specimen, a left maxilla bearing P³–M² (third upper premolar to second upper molar), associated mandible with P₄–M₃ (fourth lower premolar to third lower molar) (Fig. 1), displaced upper and lower incisors, and displaced right M¹ and M², was preserved in a fine-grained, calcareous, coprolitic nodule.

Horizon and locality. From the ~7-m-thick lowest exposed level (the Bayan Ulan beds) of the Nomogen Formation, on the northern flank of the Holy Mesa (also known as the Nomogen Mesa)⁶, about 43°20' N, 111°45' E.

Age. The pre-Neogene mammal-bearing sequence of central Asia is largely unconstrained radioisotopically, its correlation to the global geological timescale being based primarily on biostratigraphic ties to the European and North American sequences^{6,7}; the Bayan Ulan deposits are no exception. Associated fauna is similar to that of the Zhigden Member and Naran Member of the Naran-Bulak Formation and the lower member of the Gashato (also known as the Khashat)^{6,7} Formation of Mongolia. The presence of *Arctostylops* and *Prodinoceras* indicates the lowest

Bayan Ulan beds to be no younger than earliest Eocene (early Wasatchian North American Land Mammal Age) and no older than latest Paleocene (Triffanian/early Clarkforkian North American Land Mammal Ages) (~52–60 Myr)^{7–9}.

Diagnosis. Exceptionally small (3.5 mm length P³–M³) rodent, with enlarged, evergrowing upper and lower incisors with two-layered enamel restricted to the anterior surface (Fig. 2); dental formula 1023/1013; upper cheek teeth slightly unilaterally hypsodont; P³ blunt, unicuspid; P⁴ molariform; M¹ the largest upper molar; P⁴–M³ somewhat triangular and transversely elongate in occlusal outline, with weak or incomplete anterior cingulum and anteriorly situated protoconule, weak posterior cingulum interrupted by large metaconule, distinct labial shelf, lack of well developed lophs and crests, and small hypocone; M₁ with rudimentary paraconid, trigonids of P₄–M₃ compressed anteropos-

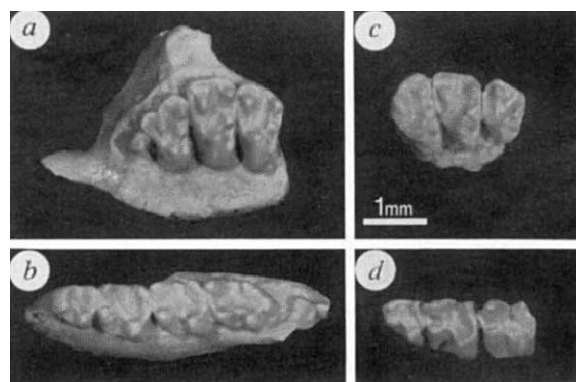


FIG. 1 Crown views of the teeth of *Tribosphenomys minutus* gen. et sp. nov.; a, left maxilla bearing P³–M² and b, left mandible containing P₄–M₃ of holotype (V10775); c, right maxilla with M¹–M³ (photographically reversed); d, left P₄–M₂ of referred specimen (V10776), associated in a second coprolitic nodule. The upper cheek tooth row shows a convex outer margin which is widest at M¹. The small P³ shows some root subdivision and a faint cingulum posterior to its blunt, central cusp. P⁴ is asymmetrically triangular in outline, with a cuspidate crown lacking crests. Its subequal metacone and paracone are separated by a deep, transversely directed cleft. This cleft becomes shallow medially, turning anteriorly to intersect the anterior edge of the tooth lingual to a small paraconule. The paracone, bearing a vertical posterior surface, and the metacone is anteroposteriorly compressed, with the latter being slightly more buccal than the former. The hypocone is small and posterior to the protocone, which forms the largest and most elevated cusp of the tooth. The metaconule is nearly as large as the metacone. The posterior cingulum is weak but more distinct than the anterior one. A moderately wide labial cingulum forms a parastylar wing anterobuccally. M¹ differs from P⁴ in its more rectangular outline, greater width, better developed labial cingulum and larger hypocone. M² is smaller than M¹, with a more lingually placed metacone bearing a little labial cingulum. M³, the smallest and squarest of the upper molars, has a tiny metacone posterobuccal to the metaconule, a very weak hypocone and no paraconule. A concave area at the ventral side of the zygomatic root, bounded by a low ridge anterolaterally, suggests that the masseter muscles were restricted to the ventral side of the zygoma. The lower teeth are more typically rodent-like than the upper teeth, being moderately elongate with well developed talonids and having anteroposteriorly compressed trigonids. Like their upper counterparts, they have sharp isolated cusps when unworn. In all but M₃ the basined talonids are markedly broader than the trigonids, the hypoconid being large and buccally projecting. A vestigial paraconid is present on unworn M₁. A low posterior arm of protoconid is lost with wear. The cristid obliqua is low, consisting mainly of a small mesoconid. Small but distinct hypoconulids are closely paired with the entoconids on P₄–M₂. The M₃ hypoconulid is robust, forming a posterior lobe. Measurement (width/length in mm): V10775, P³, 0.38/0.30; P⁴, 1.22/0.82; M¹, 1.56/0.86; M², 1.45/0.78; P₄, 0.73/0.74; M₁, 0.99/0.90; M₂, 0.98/0.95; M₃, 0.88/1.17; and V10776, M¹, 1.50/0.78; M², 1.43/0.80; M³, 1.12/0.72; P₄, 0.80/0.74; M₁, 1.01/0.91; M₂, 0.98/0.92.

teriorly, and distinct hypoconulids, particularly on M_3 ; the posterior edge of the zygomatic root is level with the metacone of M^1 . *Tribosphenomys* differs from *Alagomys*¹⁰ in having a pronounced labial shelf, relatively larger metaconule, stronger hypocone, paraconid on M_1 and a posterior arm of the protoconid on M_1 – M_2 . It is unique among rodents in having a lower molar paraconid and a shelf-like crested labial cingulum on P^4 – M^2 , giving these teeth a primitive appearance.

Although its dental formula, incisor modifications and 'squirrel-like' lower cheek teeth are typical of early rodents, the upper teeth of *Tribosphenomys* show few of the features common to nearly all other members of the order. Its primitive triangular arrangement of principal crown cusps (tribospheny) and the retention of many additional plesiomorphic features described in the diagnosis (see also Figs 1 and 2) suggest a phylogenetic position of *Tribosphenomys* peripheral to that of other rodents; this is crucial for establishing the primitive character conditions for the Rodentia. The only rodents displaying a molar crown pattern similar to the arrangement hypothesized to be primitive for Eutheria are *Tribosphenomys*, recently described but extremely fragmentary Asian forms¹⁰, another Asian species¹¹ and a North American form now under separate study (by M.R.D.); this further confirms a tribosphenic stage in rodent evolution^{12,13}.

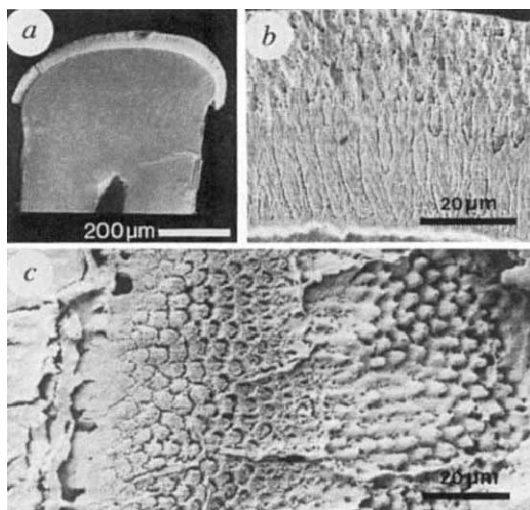


FIG. 2 Scanning electron micrographs of enamel structures of the left upper incisor from *Tribosphenomys minutus* gen. et sp. nov. (V10775). a, Cross-sectional view showing distribution of enamel; b, close-up view of the same cross-section; c, tangential (frontal) view of the internal enamel layer. The lateral surface of the tooth is towards the left in a and b and upward in c. The apical tip of the tooth is towards the right in c and outward from the page in a and b. The incisor is transversely compressed, with enamel covering the anterior surface and wrapping slightly on to its lateral and medial sides. The enamel 'cap' is flat anteriorly, and forms the widest part of the tooth. The enamel, composed of two layers, is ~50 µm thick. Crystallites lie nearly parallel to the prisms in the internal layer; prisms are arranged perpendicular to the plane of the enamel–dentine junction. Crystallites are indistinct in the external enamel layer. Prisms and interprismatic matrix are not separated near the enamel–dentine junction. Closer to the external layer, prisms become partially differentiated from the surrounding interprismatic matrix, but they are not completely separated from the latter in any region. Prisms are rounded in cross-section. Decussation of prisms is not distinct and Hunter–Schreger bands are not seen in either cross-section or tangential section. Although this arrangement does not precisely match the enamel pattern typically termed pauciserial, because of lack of Hunter–Schreger bands, structural details support the notion that pauciserial rather than multiserial enamel characterized rodents primitively, a question of considerable recent debate^{26,27}.

The parsimony analysis in Fig. 3 combines evidence from the new primitive rodents from central Asia with results of earlier palaeontological^{10,14–17} and neontological^{3,18,19} studies, yielding the first phylogenetic hypothesis of early gliriform diversification fully integrating fossil and recent data. Despite the highly plesiomorphic cheek teeth of *Tribosphenomys*, our analysis of relationships among gliriform eutherians confirms monophyly of both Glires and Rodentia, contradicting certain molecular results^{20,21}. Further results include the previously unsettled paraphyly of 'Euryomyidae' and 'Mixodontia'^{1,16,22}, support for a sister-group relationship between mimotonids and lagomorphs, and the affiliation of 'euryomyids' and rodents^{1,23}, a matter of considerable recent dispute^{16,24,25}. These results provide a basis for constraining several recently debated hypotheses concerning primitive character conditions near the base of the Rodentia, such as enamel microstructures^{26,27}, molar hypocones²⁸ and talonid condition^{13,28}. These results also reveal previously unappreciated complexity in the sequence of character transformation during early Glires evolution. A notable example concerns the disposition of the optic foramina. In rodents these structures form separate, widely spaced openings, a condition generally assumed to represent a retained eutherian plesiomorphy²³. By contrast,

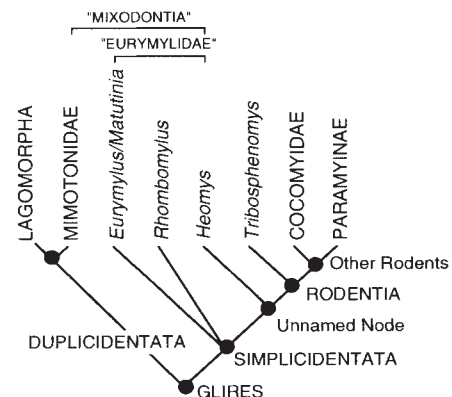


FIG. 3 Proposed phylogenetic relationships of *Tribosphenomys minutus* gen. et sp. nov., and characters diagnosing Glires and its internal nodes. This is based on a numerical cladistic analysis³⁰ of 43 anatomical characters in 13 terminal taxa detailed in a manuscript in preparation, in which eutherians including carnivorans, lipotyphlan insectivores, primates and several extinct lineages were used as outgroups. Topology represents a strict consensus of eight shortest trees (consistency index, 0.62; retention index, 0.78). Unambiguous synapomorphies supporting the various nodes in the consensus topology include the following (synapomorphies that may occur at more than one node are indicated in parentheses, assuming accelerated transformation). Glires: Enamel restricted to the anterior surface of dl^2 , l_1 absent, dl^2 large and evergrowing, dl^2 large, and evergrowing, P_2 absent, incisor foramina elongate, orbits shifted anteriorly relative to cheek teeth¹, anterior zygomatic root aligning with M^1 (l^1 absent, canines absent, tooth crown unilaterally hypsodont, molar paraconids reduced or lost, infraorbital canal short^{1,25}). Duplicidentata: Incisive foramina greatly elongate^{1,31}, ventral process of anterior zygomatic arch present²⁵, zygomatic fossa for masseter lateralis present²⁵, projection of frontal present between the premaxilla and maxilla³¹, (dl^3 posterior to dl^2). Simplicidentata: Single pair of upper incisors¹⁶, P^2 absent, glenoid fossa longitudinally elongate¹, diastema of lower jaw shorter than that of upper jaw²³, incisive foramina moderately elongate (incisor enamel two layers¹). Unnamed node: (P^4 hypocone small or absent). Rodentia: P_3 absent^{1,19,28}, P_3 small and single cusped²⁸, weak posterior arm of the protocone lacking shearing facet¹³, low cristid obliqua represented by mesoconid¹³. Other rodents: Anterior zygomatic root aligning with P^4 (upper molars quadrate; M_3 hypoconulid small). Cocomyidae and Paramyinae are used to reflect a basal dichotomy of 'other rodents' proposed by others³², although we caution that intraordinal relationships of rodents may yet prove more complicated. The non-monophyly of the traditional groupings, Euryomyidae^{16,23} and Mixodontia^{16,22}, is indicated by quotation marks.

lagomorphs, eurymylids and cocomyids are characterized by optic foramina closely aligned at the skull midline, forming a single continuous opening, an unquestionably derived condition. Placement of eurymylids as a paraphyletic sequence of outgroups to the Rodentia (Fig. 3) dictates that the condition seen in all living and most fossil rodents is not a plesiomorphic retention, but instead represents an evolutionary reversal.

Rodents are first known from many localities of latest Paleocene–earliest Eocene age in Asia and North America^{14,17,29}. They are widely considered to have originated in Asia^{1–3,10,23,29}, based on the occurrence there of eurymylids, their perceived nearest relatives. *Tribosphenomys*, now the best known example of an emerging suite of highly plesiomorphic early Cenozoic rodents¹⁰, confirms an Asiatic origin for the Rodentia, but suggests that such groups as the North American paramyids and the Asiatic ctenodactyloids are well removed from basal positions within rodent phylogeny, although this is the conventional placement of these groups^{2,14}.

An important recent review¹⁶ of the Mixodontia (that is, eurymylids and mimotonids) concluded with the prediction that "... when the diversity of mixodonts is better known the transition between them and probably both rodents and lagomorphs will be demonstrable". The discovery of *Tribosphenomys* provides precisely such a 'linking' taxon, and should help to elucidate the origin of the Rodentia, an order that has the most species among living mammals. □

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Selection by sexual conflict for evenly spaced offspring in blue tits

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In animals with parental care, parents rearing offspring of variable ages are typically assumed to exert less effort than those rearing even-aged offspring. This is because spaced births spread out peak loads in the combined food demands of all offspring^{1–3}. Creating a mixed-size sibship also helps establish a hierarchy among the young which reduces the costs of sibling rivalry⁴ and can help efficient elimination of young if food becomes short^{5,6}. We manipulated hatching spread within broods of the blue tit *Parus caeruleus* and studied postbreeding survival rate of the adults. We report here that, contrary to current theory, female parents suffer less when the young are even-aged than when they are of variable ages, whereas the opposite result was found for male parents. Apparently, the male contributes more in synchronous broods, thus lightening the female's total investment burden. In blue tits this sexual conflict over hatching pattern is won by the female because she alone incubates. By delaying incubation until most eggs have been laid, she reduces hatching span.

Most experimental studies on birth spacing have been conducted with birds, yet possible long-term costs to parents have never been investigated⁷. Though entire broods usually hatch over a few days or less⁸, even short birth intervals may have

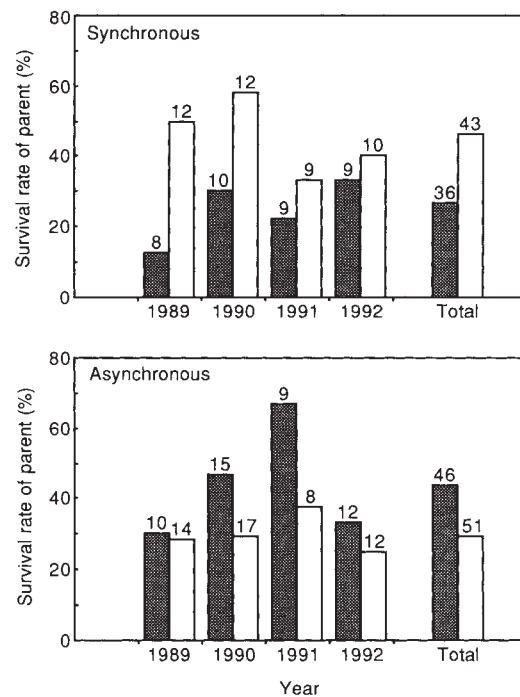
severe effects on the competitive ability of individuals within the brood. For instance, in some bird species the first-hatching chick actively kills the second sib⁹. Hence, a study of costs and benefits of avian hatching patterns may help understanding birth spacing in other animals, including humans¹⁰. We studied blue tits breeding in a woodland near Oslo, Norway, during a four-year period. These tits are resident year-round, breed readily in nestboxes, and are monogamous and single-brooded.

Adult survival rate, recorded as the percentage of birds found breeding in the subsequent year, was similar for males (35%, $n=82$) and females (37%, $n=94$) when all broods are combined; it was also similar for synchronously (37%, $n=79$) and asynchronously manipulated broods (36%, $n=97$) when the sexes are combined. However, manipulation of hatching spread had strikingly different effects on the two sexes. Females provided with synchronous broods had a higher subsequent survival rate than those provided with asynchronous broods. The opposite result was found for males (Fig. 1). Thus, females benefited from synchronous hatching, whereas males benefited from asynchronous hatching. Because males and female partners live under similar environmental conditions, it is hard to explain the sexual difference in survival from causes other than the manipulation of hatching spread itself. According to current hypotheses^{1,4,7,11–13}, parents expend less effort with asynchronous than with synchronous hatching. However, we found that the females suffered from asynchronous hatching.

Why should males and females differ in their response to brood treatment? Because the survival rate for the sexes combined was similar in synchronous and asynchronous treatments, hatching asynchrony altered the relative burden on each sex but apparently not the total parental cost involved. This suggests sexual conflict over parental care, where one parent may try to minimize investment at the expense of its mate¹⁴. We analysed male and female parental investment in their individual off-

FIG. 1 Survival rates of male (filled bars) and female (empty bars) blue tit parents after rearing broods with synchronous (top) or asynchronous hatching (bottom). Sample sizes are indicated above bars. In each of the four years, more female than male parents survived if they had been rearing a synchronous brood; the opposite pattern was found if they had been rearing an asynchronous brood. Assuming survival rate to have been independent of sex and treatment, the probability of obtaining this result by chance was only $0.5^8 = 0.004$, giving $P = 0.008$ in a two-tailed binomial test. In males, survival rate was lower for synchronous than for asynchronous experimental broods (25% versus 43%); in females, the opposite pattern was found (47% versus 29%). These differences were nearly statistically significant (males: $\chi^2 = 3.02$, d.f. = 1, $P = 0.083$; females: $\chi^2 = 2.92$, d.f. = 1, $P = 0.088$). In some cases, one of the adults was absent late in the nestling period, probably due to mortality from predation, causing starvation and heavy mortality of nestlings. Because adult survival rate was correlated with such late nestling mortality, we made a logistical regression analysis to test for the separate effect of brood manipulation on adult survival rate. Then the influence of manipulated hatching pattern on parental survival rate was significant in females (hatching pattern: $\chi^2 = 4.94$, d.f. = 1, $P = 0.026$; rate of nestling mortality between day 14 and fledging: $\chi^2 = 5.13$, d.f. = 1, $P = 0.024$), but not in males (hatching pattern: $\chi^2 = 1.67$, d.f. = 1, $P > 0.1$; rate of nestling mortality between day 14 and fledging: $\chi^2 = 2.81$, d.f. = 1, $P = 0.094$).

METHODS. Hatching spread was manipulated to be either less ($\bar{x} = 0.6$ days, s.d. = 0.2, $n = 49$; synchronous broods) or greater ($\bar{x} = 3.4$ days, s.d. = 0.5, $n = 56$; asynchronous broods) than usual but still within the range for unmanipulated broods ($\bar{x} = 2.1$ days, s.d. = 1.0, range 0–5 days, $n = 103$). This was done by interchanging newly hatched young between nests without changing brood size. No significant differences existed between the two treatment groups in time of egg laying, clutch size, number of young hatched, or in initial hatching spread (*t*-tests, $P > 0.09$). Mean body mass when 14 days old for young that fledged was lower for synchronous ($\bar{x} = 10.3$ g, s.d. = 1.2, $n = 44$) than for asynchronous experimental broods ($\bar{x} = 10.7$ g, s.d. = 0.8, $n = 53$; *t*-test, $t = 2.16$, d.f. = 95; $P = 0.033$; no year effects: one-factor ANOVA, $F = 2.45$, d.f. = 3, 93; $P = 0.068$). However, a similar number of young fledged from the synchronous ($\bar{x} = 6.6$, s.d. = 3.3, $n = 49$) and asynchronous broods ($\bar{x} = 7.0$, s.d. = 2.9, $n = 56$; two-factor ANOVA: brood treatment, $F = 0.06$; d.f. = 1, 97; $P > 0.8$; year, $F = 18.44$; d.f. = 3, 97;



$P < 0.001$; interaction, $F = 0.41$; d.f. = 3, 97; $P > 0.7$), and a similar number of recruits were found in the subsequent local breeding population from the synchronous ($\bar{x} = 0.25$, s.d. = 0.48, $n = 49$) and asynchronous broods ($\bar{x} = 0.30$, s.d. = 0.54, $n = 56$; *t*-test, $t = 0.59$, d.f. = 103, $P > 0.5$; no year effects: one-factor ANOVA, $F = 0.61$; d.f. = 3, 97; $P > 0.8$). The adults were colour-ringed for individual recognition during the nestling period, and survival rate was recorded as the percentage of birds found breeding in the subsequent year. In the analyses of adult survival rate, only nests with at least one young fledged are included.

spring. In some birds, females spend more effort than males in feeding the youngest, smallest offspring of the brood^{15–19}. In the present study, we found the same sexual difference in tit provisioning when volant young were 20–26 days old. We followed 11 families for one hour, and in six cases both parents were observed feeding identifiable offspring. Using the body weights recorded when the young were 14 days old, females fed smaller offspring than males (paired *t*-test, $t = 2.61$, d.f. = 5, $P = 0.048$). In five cases we located the young that had been lightest before leaving the nest. In each case the female parent alone was feeding this smallest young.

These data suggest that males assume responsibility for the larger offspring. The body mass of the heaviest nestling on day 14 post-hatch was greater for asynchronous than for synchronous broods ($t = 2.82$, d.f. = 95, $P = 0.006$). Thus, in the case of asynchronous broods, males were likely to work less after the young fledged, presumably as reflected in elevated male survival rate. If male survival rate is positively related to the size of the largest offspring, then we would expect the body mass of the largest offspring in broods where the male parent survived until the subsequent breeding season to be higher than the body mass of the largest offspring in broods where the male parent did not survive. This prediction was supported ($t = 2.59$, d.f. = 80, $P = 0.011$). By contrast, females appear to increase their level of investment in order to keep less competitive, late-hatching nestling alive. That effort may have considerably reduced female survival rate. The more nestlings that died, the fewer females survived (see Fig. 1 legend).

Why did males and females differ in their pattern of food provisioning? Offspring sired through extra-pair fertilizations are common in birds²⁰, including blue tits²¹. It has been suggested

that late-hatched young are more often sired through cuckoldry than earlier-hatched young and thus have a lower value to the resident male than to the female parent^{17,22}. However, this has not yet been studied in blue tits. In many bird species²³, including tits^{21,24,25}, males tend to have a higher annual survival rate than females. Hence, a fundamental difference may exist between the sexes in the priority of parental investment in current versus future reproduction, and so between caring for small, often undernourished young with lower survival prospects^{26,27} on one hand, and on territorial defence and moulting on the other hand. During breeding, males give higher priority to moult than females in blue tits²⁸ and in many other bird species²⁹. All of these sources of sexual conflict may force females to provide extra care for last-hatched young.

Passerines hatch their brood over a few days or less^{7,8}, but current theory emphasizes adaptive advantages to asynchronous hatching. Blue tits lay a clutch of about 10 eggs at the rate of one egg per day. Hence, if incubation had started with the first egg laid, hatching would take about 9 days. We found a mean hatching span of only 2 days. Because females in blue tits and in some other species often assume care of the smallest or youngest offspring, they may regulate incubation to ensure synchronous hatching. Asynchrony favours males, presumably because they assume care of older, well developed young which demand less post-fledging care. But asynchrony impairs female survival, presumably because the smaller young they care for require substantially more investment. Thus our results demonstrate sexual conflict over birth spacing in blue tits in which synchronous hatching improves survival of the female parent but impairs survival of her mate. This conflict, however, is won by the female because she alone is responsible for incubation. □

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Field trial of a genetically improved baculovirus insecticide

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IMPROVEMENT of biological pesticides through genetic modification has enormous potential and the insect baculoviruses are particularly amenable to this approach^{1,2}. A key aim of genetic engineering is to increase their speed of kill, primarily by the incorporation of genes which encode arthropod or bacterially derived insect-selective toxins^{3–11}, insect hormones^{12,13} or enzymes^{14,15}. We report here the first, to our knowledge, field trial of a genetically improved nuclear polyhedrosis virus of the alfalfa looper, *Autographa californica* (AcNPV) that expresses an insect-selective toxin gene (AaHIT) derived from the venom of the scorpion *Androctonus australis*^{16–18}. Previous laboratory assays with the cabbage looper, *Trichoplusia ni*, demonstrated a 25% reduction in time to death compared to the wild-type virus, but unaltered pathogenicity⁶ and host range¹⁹. In the field, the modified baculovirus killed faster, resulting in reduced crop damage and it appeared to reduce the secondary cycle of infection compared to the wild-type virus.

The trial was designed to test the genetically modified virus under challenging conditions where insect density was high and the larvae were large and capable of causing serious crop damage. The genetically modified virus (AcST-3)⁶ and parent clone (AcNPV C6) were compared at low, medium and high doses,

which were applied as a spray against third instar *T. ni* on cabbage. Laboratory data suggested that peak AcST-3 mortality should occur earlier than that caused by the wild-type virus⁶. Four samples were taken: two before either virus caused insect death (and release of secondary inocula), one between the two peaks of virus mortality and a final sample after both virus epizootics had occurred, to measure crop damage and larval survival.

Virus treatment significantly reduced insect damage compared to the untreated controls (Fig. 1a). Untreated larvae caused a 22% reduction in leaf area, whereas the loss in virus-treated plots was only 12.5% (mean leaf area 183 cm² ± 5 s.e.). Recombinant-treated plots had significantly less crop damage than those treated with the unmodified virus (Fig. 1b, c). Insects infected with the recombinant virus consumed 29% and 23% less than the equivalent wild-type treatment at low and medium plus high doses, respectively. This reduction in leaf damage was clearly a result of the earlier death of recombinant-infected insects: larvae died 10–15% earlier than insects infected with the wild-type (Table 1). This reduction was not as large as that reported in laboratory studies⁶. This could be the result of numerous factors which influence baculovirus pathogenicity, including insect age, method of dosing, food plant and environmental variables. Toxin expression by the recombinant virus induces larval paralysis⁶: in the field this resulted in many larvae falling off the plants before death (for example at the AcST-3 high dose (day 11), 62% of larvae were found paralysed/dead on the soil, compared to none in wild-type treatments). The earlier peak in recombinant-induced paralysis/mortality is clearly illustrated by comparison of the number of live larvae recovered at each sampling date in the high-dose treatment (Fig. 2). There was no difference between treatments at 2 and 7 days after spraying. However, by day 11, peak mortality had already occurred in the recombinant treatment but not in the wild-type treatment (Fig. 2). By day 16, the number of larvae collected from the wild-type treatment had also declined.

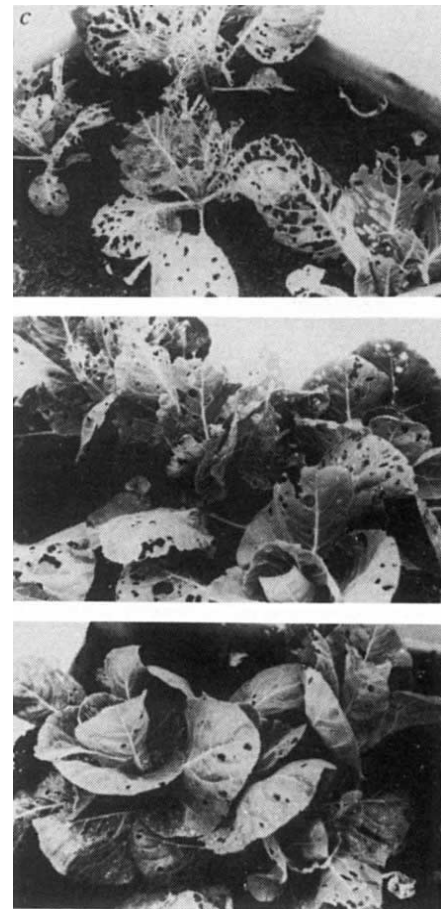
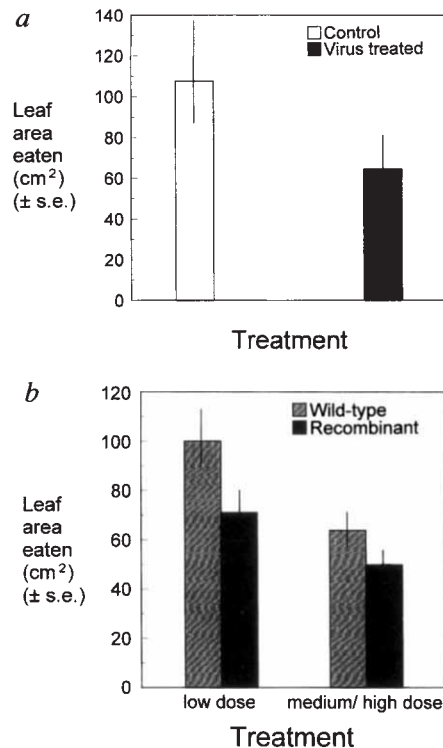
The patterns of virus-induced mortality obtained in the trial were more complex. Overall recovery of live larvae was high with 10,442 larvae recovered of the 14,000 released (74.6%).

TABLE 1 Mean time to death (days ± s.e.) for *T. ni* Larvae

Sample time and size	Wild-type virus dose (polyhedra per m ²)			Recombinant virus dose (polyhedra per m ²)		
	10 ⁶	10 ⁷	10 ⁸	10 ⁶	10 ⁷	10 ⁸
day 2	8.0 (±0.15)	7.5 (±0.15)	7.3 (±0.14)	7.0 (±0.14)	6.4 (±0.13)	6.2 (±0.12)
sample size =	122	369	468	131	358	444
day 7	12.6 (±0.25)	11.7 (±0.23)	10.9 (±0.21)	11.3 (±0.22)	10.1 (±0.20)	9.5 (±0.19)
sample size =	224	396	410	177	293	401

Mean time to death for larvae collected from the trial at different times after virus application. Treatments and sampling regime as described for Fig. 1. On each sampling occasion five cabbages were taken from each plot and the *T. ni* larvae removed and counted. They were then returned to the laboratory and were reared individually on artificial diet²³ at 24 °C ± 1 °C and checked daily for death or pupation. Viral deaths were usually identified by eye. Suspicious deaths were smeared, stained with Giemsa and diagnosed using oil-immersion light microscopy at ×1,000. Only those larvae collected from the first two sampling dates were used in the analysis because insects collected at the two later time points could have become infected from either the initial or secondary inoculum (see text). Time to death was analysed using the Weibull distribution which allows the hazard function to vary with time. Recombinant virus-infected larvae died faster than wild-type virus-infected larvae on all sampling occasions ($F = 20.48$; d.f. = 4, 71; $P < 0.001$). Speed of kill also increased with dose on all sampling occasions ($F = 12.23$; d.f. = 8, 71; $P < 0.001$).

FIG. 1 Mean leaf areas eaten ($\text{cm}^2 \pm \text{s.e.}$) per cabbage (total of three leaves) in infested control and virus-treated plots at 16 days after virus application. Twenty cabbage seedlings were planted within 1 m^2 plots on the University Farm, Wytham, UK, on August 2nd 1993. Plots were caged in fine mesh netting 1 m high and buried to a depth of 10 cm. Soil between the plots was treated fortnightly with glyphosate herbicide until virus application. Four weeks after planting, 500 third-instar *T.ni* larvae were introduced to each plot (25 per plant) from a laboratory culture. Wild-type and recombinant virus were applied at 10^6 , 10^7 and 10^8 polyhedra per m^2 using a handheld, ULV spinning-disc sprayer (11,000 r.p.m., 50 ml min^{-1}). Plots were lined during spraying to eliminate drift. Tween 20 (1%) was added to the purified virus before application. Controls with and without insects were also set up and treated with 1% Tween 20 in water. Each treatment had four replicates. Plots were randomly sampled at 2, 7, 11 and 16 days after spraying. On the last sampling occasion the leaf areas of three leaves from each of the remaining five cabbages were measured using a leaf area analyser. *a*, Leaf areas eaten in control and virus-treated plots. Error bars represent the least significant difference between control and virus-treated plots. Virus-treated plots sustained significantly less damage ($F=5.86$; d.f. = 1,26; $P<0.05$; ANOVA on $\log_e(\text{area eaten})$ with contrasts and normal errors). *b*, Leaf areas eaten in wild-type and recombinant virus-treated plots. Model simplification resulted in medium and high doses being grouped together ($F=0.26$; d.f. = 1,20; n.s.). The recombinant-treated plots sustained significantly less damage ($F=4.34$; d.f. = 1,21; $P<0.05$), and the low-dose sustained significantly more damage ($F=$

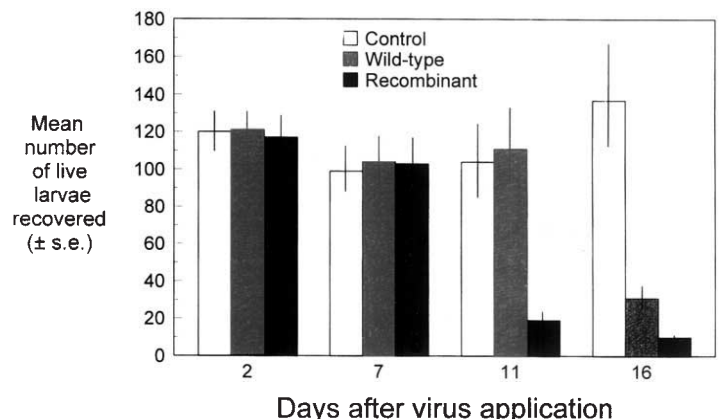


8.01; d.f. = 1,21; $P<0.05$). *c*, Damage to cabbage plants in (top) infested control, (middle) high-dose wild-type and (bottom) high-dose recombinant-virus treatments at the final timepoint.

Recovery from control plots was even higher (92%), indicating low levels of natural mortality. Non-virus mortality in the larvae recovered was $<5\%$ in the controls and $<1\%$ in the treated plots. As expected, there was a significant increase in mortality with increasing virus dose, with over 95% mortality at the highest dose in both treatments. On the first two sampling occasions there was no difference in mortality between virus treatments, indicating that they did not differ in pathogenicity (Fig. 3*a, b*). However, on the last two sampling dates the recombinant virus caused significantly less mortality (Fig. 3*c, d*). These samples

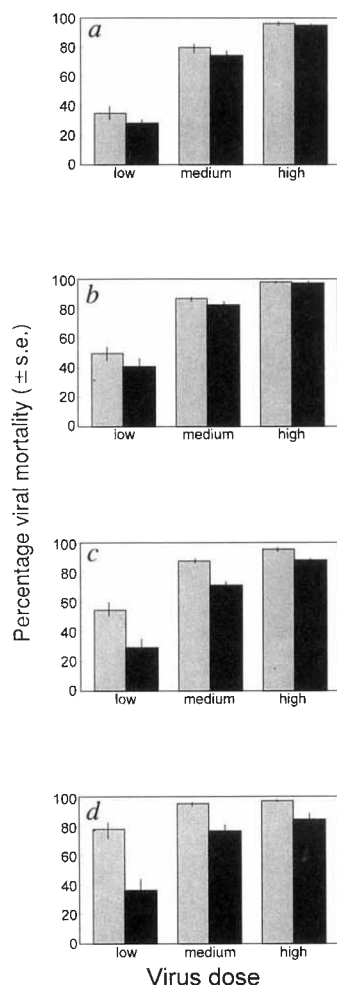
are likely to be biased, because of the loss of paralysed/dead larvae in the recombinant-treated plots, which would lead to an underestimate of mortality. However, the number of survivors (comparable between treatments because an equal number of larvae were released initially) followed a similar pattern: there were more survivors in the recombinant-treated plots at day 16 ($\chi^2=20.25$, $P<0.01$, Poisson errors, scale parameter 1.87). As this difference in survivorship occurs only at the last timepoint, it suggests that it may arise from variation in secondary transmission (release of virus from larvae killed by the original

FIG. 2 Mean number ($\pm \text{s.e.}$) of live *T.ni* larvae recovered from controls and high-dose wild-type and recombinant-virus treatments on four separate sampling occasions. For 2 and 7 days after application, there was no significant difference in the numbers recovered from the three treatments ($F=0.45$; d.f. = 2,25; n.s.; GLIM with Poisson errors and a scale parameter of 3.6; and $F=0.75$; d.f. = 2,25; n.s., GLIM with Poisson errors and a scale parameter of 5.7, respectively). Eleven days after application, recovery differed between treatments ($F=6.53$; d.f. = 2,25; $P<0.01$, GLIM with Poisson errors and a scale parameter of 15.2); significantly fewer larvae were recovered from the recombinant-treated plots. For this timepoint, the model may be simplified to recombinant plots versus other plots (that is, control and wild-type plots combined) without significant loss of explanatory power ($F=0.16$; d.f. = 1,25; n.s., GLIM with Poisson errors and a scale parameter of 15.2). On the final sampling occasion the three treatments are significantly different ($F=10.3$; d.f. = 2,25; $P<0.01$, GLIM with Poisson errors and a scale parameter 20.3). Retrieval is at its lowest in the recombinant-treated plots (recombinant versus wild type: $t=2.52$; d.f. = 25; $P<0.02$) and is at its highest in the control plots (control versus wild type: $t=2.69$; d.f. = 25;



$P<0.02$). Model checking involved inspection of residuals and a normal order statistical plot.

FIG. 3 Percentage virus-induced mortality in wild-type (shaded bars) and recombinant (black bars) treatments at three virus doses, sampled on four occasions: a, 2 days; b, 7 days; c, 11 days, and d, 16 days after release. Error bars are the least significant difference between treatments. Each timepoint was analysed separately. For the first two timepoints, mortality varied between doses (day 2; $F=155.2$; d.f. = 2,20; $P<0.001$; ANOVA with binomial errors and scale parameter 2.98; day 7; $F=154.8$; d.f. = 2,20; $P<0.001$, ANOVA with binomial errors and scale parameter 2.39), but mortality did not differ significantly between virus treatments (day 2; $F=3.04$; d.f. = 1,20; n.s.; ANOVA as above; day 7; $F=3.97$; d.f. = 1; n.s.; ANOVA as above). However, at days 11 and 16, both virus type and dose were significantly different, with the recombinant virus causing significantly lower mortality (day 11: virus type: $F=30.28$; d.f. = 1,20; $P<0.01$; dose: $F=73.8$; d.f. = 2,20; $P<0.01$; Day 16, virus type: $F=41.33$; d.f. = 1,20; $p<0.01$; dose: $F=17.29$, d.f. 2,20; $p<0.01$). In each case the analysis is weighted according to the sample size, so allowing for the difference in recovery rates illustrated in Fig. 2.



sprayed inoculum). This variation may be due to differences in the pathology and behaviour of insects infected by the two viruses. Insects infected with wild-type NPV usually remained on the plant after death, where they liquified and thus aided release of large quantities of virus, whereas recombinant infected larvae fell onto the soil and did not lyse. The yield of virus was also reduced in larvae infected with the recombinant (such as 9.6×10^8 polyhedra/larva (C6) and 9.9×10^7 polyhedra/larva (AcST-3) for *T.ni* larva infected as fourth instars), further reducing the likelihood of transmission. Thus the recombinant virus may be less readily transmitted to other larvae, resulting in reduced secondary infection, which, if confirmed, could have important implications for risk assessment. The environmental safety of genetically modified baculoviruses has been discussed²⁰, particularly regarding host range^{21,22}; these results indicate that evaluating the safety of such organisms requires the integration of several factors, including transmissibility, environmental persistence and host range to arrive at a full risk assessment.

In summary, we have demonstrated that a genetically improved baculovirus insecticide expressing an insect-selective toxin kills *T.ni* larvae more rapidly in the field than the wild-type virus, resulting in improved crop protection. There was also evidence that secondary transmission was lower in insects treated with recombinant virus. These results highlight the importance of speed of action in achieving reduced crop damage and demonstrate that genetic modification can improve the efficacy of bio-insecticides in the field. □

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Correlated neuronal discharge rate and its implications for psychophysical performance

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SINGLE neurons can signal subtle changes in the sensory environment with surprising fidelity, often matching the perceptual sensitivity of trained psychophysical observers^{1–10}. This similarity poses an intriguing puzzle: why is psychophysical sensitivity not greater than that of single neurons? Pooling responses across neurons should average out noise in the activity of single cells, leading to substantially improved psychophysical performance. If, however, noise is correlated among these neurons, the beneficial effects of pooling would be diminished^{10–12}. To assess correlation within a pool, the responses of pairs of neurons were recorded simultaneously during repeated stimulus presentations. We report here that the observed covariation in spike count was relatively weak, the correlation coefficient averaging 0.12. A theoretical analysis revealed, however, that weak correlation can limit substantially the signalling capacity of the pool. In addition, theory suggests a relationship between neuronal responses and psychophysical decisions which may prove useful for identifying cell populations underlying specific perceptual capacities.

We obtained data from 100 pairs of neurons in the middle temporal visual area (MT, or V5) while three rhesus monkeys viewed random dot patterns presented on a video monitor. Because directionally selective neurons in MT supply signals for discriminating the direction of motion in these displays^{10,13–15}, this system provides an ideal opportunity to measure correlation within a pool of sensory neurons contributing to psychophysical performance. Action potentials were recorded using a single electrode and a spike sorting device which recognized individual

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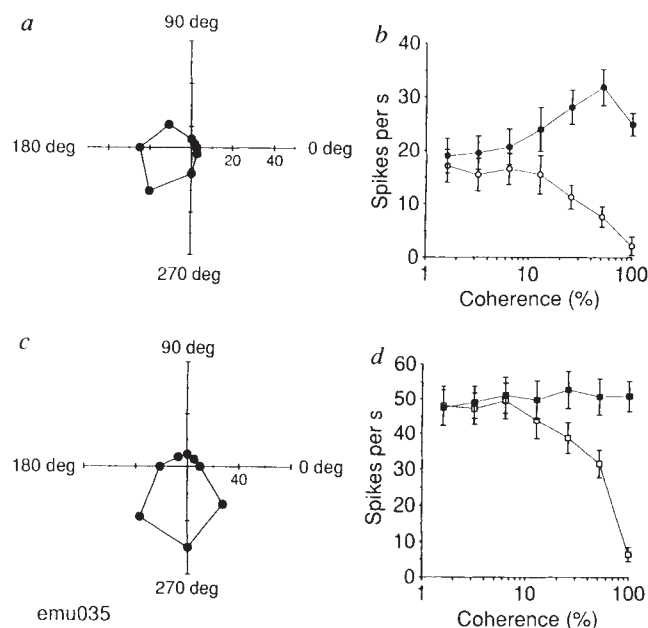


FIG. 1 Average responses of a pair of simultaneously recorded MT neurons during the fixation and discrimination tasks. Polar plots (a, c) are direction tuning curves measured during the fixation task for neuron 1 (a, b) and neuron 2 (c, d). For each data point, the angle indicates the direction of stimulus motion and the distance from the origin represents the average response (spikes per s) to that direction. Coherence-response functions (b, d) depict average responses to motion signals of increasing strength. Empty symbols indicate responses to preferred direction motion and filled symbols show responses to the opposite (null) direction. Error bars denote standard deviations. METHODS. Our methods have been described previously¹⁰. To summarize, direction tuning curves were measured with moving random dot patterns. The position of the dot pattern and the motion speed were set to stimulate the receptive fields of both neurons as well as possible. In the discrimination task, a specified percentage of dots in the visual display moved coherently in one direction while the remaining dots were plotted briefly at random locations, creating a masking motion noise. Monkeys received liquid rewards for reporting the direction of coherent motion correctly. Task difficulty was varied by changing the percentage of dots in coherent motion. On each trial, the direction of motion was either the preferred or null direction of the recorded neurons. If the preferred directions of the two neurons differed substantially, the axis of discrimination was set to the preferred-null axis of the best-responding neuron. In both the discrimination and fixation tasks, each stimulus condition was repeated 10–40 times in random order. Both discrimination and fixation data were obtained for 42 pairs of neurons; fixation data alone were obtained for the remaining pairs.

waveforms using an eight-point template-matching algorithm¹⁶. We accepted data only from experiments with excellent separation between the two templates and between each template and the background noise.

Responses were recorded during two behavioural tasks, fixation and discrimination. In the former, the monkeys maintained fixation while the visual stimulus was presented within the receptive fields of the two cells. We measured the directional tuning of each neuron and used these data to assess the degree of correlated responsiveness. In the discrimination task, the monkeys reported the direction of coherent motion embedded in a field of random motion noise (see Fig. 1 legend). These data allowed us to assess correlated responsiveness during performance of the discrimination task. Figure 1 illustrates the responses of a pair of MT neurons in the two tasks. Both cells responded selectively to the direction of coherent motion¹⁷, but signalled direction with decreasing reliability as coherent motion was reduced¹⁰.

For each pair, we computed the correlation coefficient, r , between the responses of the two neurons to multiple presentations of a given stimulus. This process was repeated for each

stimulus condition tested. Throughout our analyses a response was considered to be the total number of action potentials recorded during presentation of a visual stimulus. A χ^2 test of homogeneity showed that the correlation coefficients computed for a pair of neurons were independent of stimulus condition in 89% of the experiments ($P > 0.05$; correlation coefficients were first transformed to Fisher's z to conform to normality assumptions¹⁸). Furthermore, we found no systematic difference in the average correlation coefficient between the two behavioural tasks (paired t -test, $P > 0.75$). We therefore combined data across stimulus conditions and tasks to compute a single correlation coefficient for each pair of neurons. To remove the influence of confounding variables such as stimulus strength, spike counts were converted to z -scores using the mean and standard deviation for repetitions of each stimulus type. We then calculated the correlation coefficient between ordered pairs of z -scores.

For the 100 pairs of MT neurons, the mean correlation coefficient was 0.12, a value significantly greater than zero (t -test, $P < 0.0001$). Furthermore, the correlation coefficient depended significantly on the difference in preferred direction of the two neurons. Figure 2a shows the distribution of correlation

FIG. 2 Frequency histograms of the correlation coefficients measured between pairs of MT neurons. a, Distribution of correlation coefficients for 52 pairs of neurons whose preferred directions differed by less than 90°. b, Distribution of coefficients for 13 pairs of neurons whose preferred directions differed by more than 90° and 35 pairs for which one neuron was not directional. Three analyses were done to control for artefactual sources of correlated responsiveness. First, we suppressed slow changes in responsiveness by renormalizing the standardized responses in blocks of 20 trials. This manipulation removed any yoked drift in overall responsiveness that might cause spurious correlation between the two neurons, but had no effect on the computed correlation coefficients (paired t -test, $P > 0.9$). To ensure that correlations were not driven entirely by outlier data points (resulting, perhaps, from brief lapses in the quality of isolation), we 'pruned' from each data set trials on which the response of either neuron was more than two standard deviations from that neuron's mean response for a given stimulus condition. Pruning did not affect the correlation coefficients (paired t -test, $P > 0.4$). Last, our random dot patterns typically are computed uniquely on each trial so that the monkey cannot solve the task by memorizing specific patterns of dots for each motion condition. This, however, introduces a small amount of variation among nominally identical stimuli that might give rise to spurious correlation. For four neuronal pairs, therefore, we obtained data using stimuli computed in the usual manner and then using a single predetermined random dot pattern for each stimulus condition (the difference in preferred directions was less than 45° for three pairs and was 103° for the remaining pair). Without stimulus-induced variance, the mean correlation coefficient remained significantly greater than 0 (paired t -test, $P < 0.005$). The correlation coefficients were similar with and without stimulus variation (means = 0.15 and 0.22, respectively), although our sample is too small to exclude the possibility of a moderate difference.

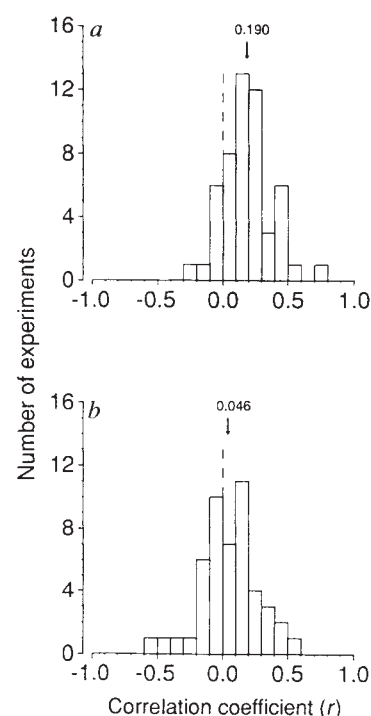


FIG. 3 Effect of pooling for different levels of correlation. *a*, Signal-to-noise ratio of the pooled average response as a function of increasing pool size. *b*, Covariation between individual responses and the pooled average response (ordinate) as a function of increasing pool size (abscissa). Each curve shows results for a particular correlation level within the pool (mean correlation, $\bar{r}=0$ to 0.5 in increments of 0.05). The dashed curves indicate the correlation levels that are most plausible from our physiological data. The curves represent analytical solutions to the equations presented below for M identically distributed neural signals. In the present context, each signal distribution represents the responses of a single neuron contributing to a sensory pool. The assumption of identically distributed signals, while overly simplified from real pools of neurons, is convenient computationally and does not influence our conclusions substantially. For *a*, we calculate the signal-to-noise ratio of M summed signals, $X_1 \dots X_M$

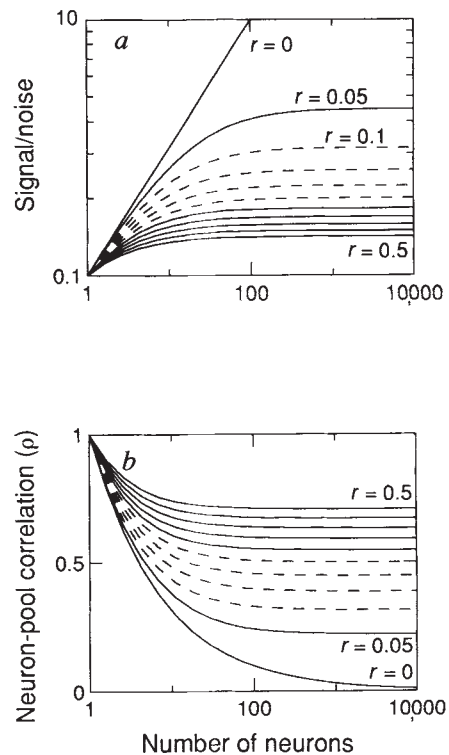
$$\begin{aligned} S/N &= \left\langle \frac{\sum_{i=1}^M X_i}{\sigma_\Sigma} \right\rangle \\ &= \frac{M\langle X \rangle}{\sqrt{\sum_{i=1}^M \sum_{j=1}^M \text{Cov}[X_i, X_j]}} \\ &= \frac{M\langle X \rangle}{\sqrt{M\sigma^2 + M(M-1)\bar{r}\sigma^2}} \end{aligned} \quad (1)$$

where σ_Σ is the standard deviation of the sum and brackets denote expectation. Each curve depicts the S/N of the pool normalized to the S/N of a single neural signal. For large M , improvement is limited by $1/\sqrt{\bar{r}}$. For *b*, we compute the expected correlation, ρ , between one random signal, X_1 , and the sum of $X_1 \dots X_M$. The covariance can be written as

$$\begin{aligned} \rho\sigma_1\sigma_\Sigma &= \rho\sigma\sqrt{\sum_{i=1}^M \sum_{j=1}^M \text{Cov}[X_i, X_j]} \\ &= \rho\sigma\sqrt{M\sigma^2 + M(M-1)\bar{r}\sigma^2} \end{aligned} \quad (2)$$

or in terms of products and their expectations

$$\begin{aligned} \text{Cov}\left[X_1, \sum_{i=1}^M X_i\right] &= \left\langle X_1 \sum_{i=1}^M X_i \right\rangle - \langle X_1 \rangle \left\langle \sum_{i=1}^M X_i \right\rangle \\ &= \langle X_1^2 \rangle + \sum_{i=2}^M \langle X_1 X_i \rangle - M\langle X \rangle^2 \\ &= \sigma^2 + \sum_{i=2}^M \text{Cov}[X_1, X_i] \\ &= \sigma^2 + (M-1)\bar{r}_1\sigma^2 \end{aligned} \quad (3)$$



where $\bar{r}_1$ is the average correlation coefficient between signal 1 and each of the other signals. Solving for ρ in equations (2) and (3) we obtain

$$\rho = \frac{1 + (M-1)\bar{r}_1}{\sqrt{M + M(M-1)\bar{r}_1}} \quad (4)$$

The correlation coefficient, ρ , between a single random signal and the sum to which it contributes is plotted in *b*. By analogy with real neuronal pools, this correlation could reflect the covariation between a single neuron's response and the outcome of a psychophysical decision governed by the summed signal, or decision variable. Even with arbitrarily large pools the correlation asymptotes at $\sqrt{\bar{r}_1}$.

coefficients for pairs of neurons whose preferred directions differed by less than 90 degrees, and Fig. 2*b* depicts the corresponding distribution for pairs that failed to meet this condition. The difference between the two distributions is highly significant (*t*-test, $P < 0.001$). The correlation values in Fig. 2*a* agree well with similar measurements made in striate cortex of cats¹² and inferotemporal cortex of monkeys¹⁹.

Our data indicate that adjacent MT neurons covary weakly in their response to visual stimuli. Even such weak covariation places fundamental limits on the signal-to-noise ratio (S/N) of any stimulus represented in the activity of a pool of sensory neurons. Figure 3*a* shows the expected improvement in S/N conferred by signal averaging among neuronal pools of increasing size. If neurons within the pool are independent ($r=0$), then S/N should improve by the square root of their number. If neurons within the pool covary even weakly, however, S/N of the pooled response is limited, reaching an asymptote at $r^{-1/2}$ times the S/N of a single neuron in the pool. Note that little increase in S/N is expected for pool sizes larger than 50–100 neurons. Thus, the beneficial effects of pooling are curtailed sharply by weak correlation, rendering more plausible the impressive sensitivity of single neurons relative to psychophysical threshold.

Because our data were recorded from adjacent neurons, we may have overestimated the average correlation in the pool if correlation declines with distance between neurons. Figure 3*a*

shows, however, that S/N would reach an asymptotic limit near 100 neurons even if the average correlation within the pool were less than our measured value.

Weak correlation can lead to a covariation between the noisy responses of single neurons and psychophysical decisions. Figure 3*b* illustrates the trial-to-trial correlation expected between the responses of any single neuron and the pooled signals represented in Fig. 3*a*. If neurons within the pool are independent, the covariation of any one neuron with the pooled response must vanish with increasing pool size ($r=0$). If pairs of neurons covary even weakly, however, each neuron retains a covariation with the pooled average even for large pools. If psychophysical decisions are based on pooled signals like those in Fig. 3, near-threshold decisions should be correlated with the response fluctuations of single neurons within the pool.

We have in fact observed such covariation between neuronal responses and psychophysical decisions during performance on our direction discrimination task^{20,21}, and a similar phenomenon has been reported in the somatosensory system²². Our analysis suggests that the covariation of single-neuron responses and psychophysical decisions, an observation that strains credulity at first glance, is a logical consequence of weakly correlated noise within the pool of sensory neurons leading to the decision. If such correlation is a general characteristic of neuronal pools underlying psychophysical performance, trial-to-trial covariation between neuronal responses and decisions may prove useful

for identifying neurons that contribute to various perceptual capacities. □

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Activation of the cloned muscarinic potassium channel by G protein $\beta\gamma$ subunits

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ACETYLCHOLINE released during parasympathetic stimulation of the vagal nerve slows the heart rate through the activation of muscarinic receptors and subsequent opening of an inwardly rectifying potassium channel¹. The activation of these muscarinic potassium channels is mediated by a pertussis toxin-sensitive heterotrimeric GTP-binding protein (G protein)^{2,3}. It has not been resolved whether exogenously applied $G_{\alpha}^{4,5}$ or $G_{\beta\gamma}^{6,7}$, or both, activate the channel. Using a heterologous expression system, we have tested the ability of different G protein subunits to activate the cloned muscarinic potassium channel, GIRK1^{8,9}. We report here that coexpression of GIRK1 with $G_{\beta\gamma}$, but not $G_{\alpha\beta\gamma}$, in *Xenopus* oocytes results in channel activity that persists in the absence of cytoplasmic GTP. This activity is reduced by fusion proteins of the β -adrenergic receptor kinase and of recombinant G_{α} -GDP,

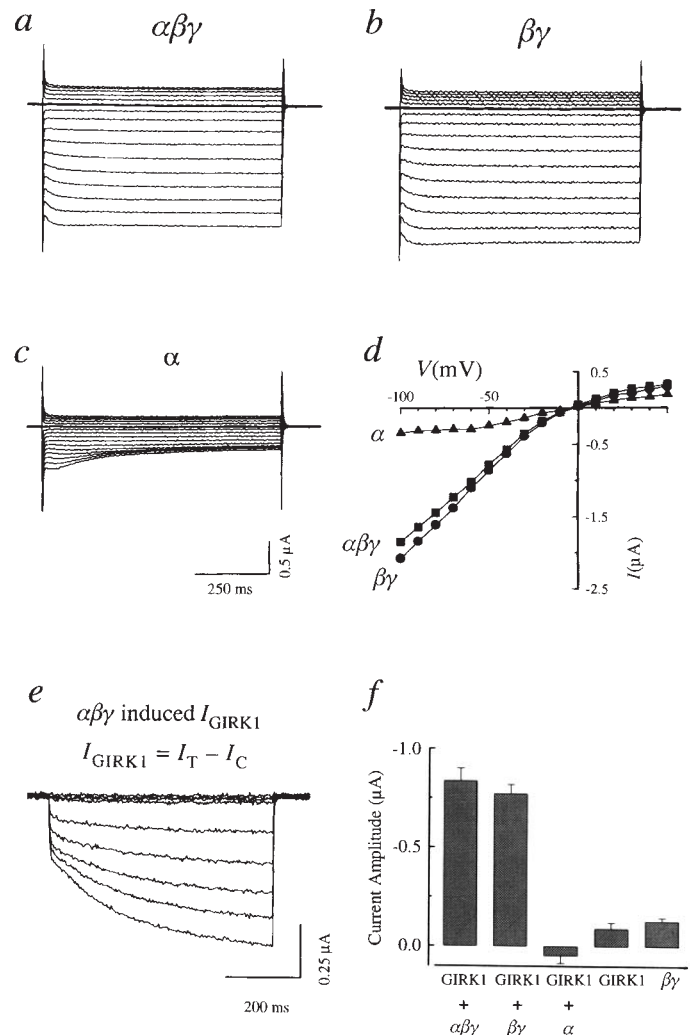


FIG. 1 Coexpression of GIRK1, $\beta 1$ and $\gamma 2$ results in channel activation. *a-c*, Inwardly rectifying potassium currents recorded under two-electrode voltage clamp from *Xenopus* oocytes injected with cRNA for GIRK1 and for the various G protein subunit combinations. *a*, $\alpha 2$, $\beta 1$ and $\gamma 2$; *b*, $\beta 1$, $\gamma 2$; or *c*, $\alpha 2$. The holding potential was 0 mV (the equilibrium potential for potassium, E_K) and current elicited by voltage pulses from -100 to +50 mV in 10 mV increments. *d*, Current-voltage (*I-V*) plot of the steady-state current for traces shown in *a* (■), *b* (●) and *c* (▲). *e*, GIRK1 current induced by $\alpha 2$, $\beta 1$, $\gamma 2$. In the same batch of oocytes, the average endogenous current present in oocytes injected with only $\beta 1$, $\gamma 2$ ($n = 9$) was subtracted after leak subtraction from the average total current in oocytes produced by coinjecting GIRK1 and $\alpha 2$, $\beta 1$, $\gamma 2$ (I_T , $n = 9$). Current traces elicited by voltage pulses from +40 to -120 mV in 20 mV increments. *f*, A bar graph of the mean current amplitudes at -100 mV from oocytes injected with cRNA for GIRK1 (-160 $\pm$ 43 nA; $n = 3$) or GIRK1 together with $\alpha 2$, $\beta 1$, $\gamma 2$ (-836 $\pm$ 62 nA; $n = 32$), $\beta 1$, $\gamma 2$ (-771 $\pm$ 47 nA; $n = 39$) or $\alpha 2$ (48 $\pm$ 38 nA; $n = 14$), or $\beta 1$, $\gamma 2$ alone (-129 $\pm$ 18 nA; $n = 10$). Current amplitudes recorded from oocytes injected with GIRK1 and $\alpha 2$, $\beta 1$, $\gamma 2$ or $\beta 1$, $\gamma 2$ were significantly larger than those from oocytes injected with GIRK1 and $\alpha 2$, GIRK1 alone or $\beta 1$, $\gamma 2$ alone (ANOVA followed by Fisher's least significance difference test (LSD), $P < 0.001$). Assuming a linear leak, the current at +20 mV was scaled and subtracted from the current at -100 mV (measured at the end of the voltage pulse) or a P/-10 protocol was used.

METHODS. Oocytes were isolated as described previously⁸, and injected with 50 nl solution containing *in vitro* transcribed (~ 100 ng μ l⁻¹) GIRK1 cRNA, with or without 150 ng μ l⁻¹ $\beta 1$, $\gamma 2$ cRNA, and 3' untranslated region of the *Xenopus* β -globin gene (gift from A. Connolly). Macroscopic currents were recorded at 22–25 °C using two-electrode voltage-clamp as described previously⁸. The bath solution contained 90 mM KCl; 2 mM $MgCl_2$; 5 mM HEPES adjusted to pH 7.4 with KOH. Average values are mean $\pm$ s.e.m.

both of which are known to interact with $G_{\beta\gamma}$ ^{10,11}. Moreover, application of recombinant $G_{\beta\gamma}$, but not $G_{\alpha 2}$ -GTP- γ S, activates GIRK1 channels. Thus $G_{\beta\gamma}$ appears to be sufficient for the activation of GIRK1 muscarinic potassium channels.

The activation of G protein-coupled receptors stimulates the replacement of GDP by GTP and the dissociation of the trimeric G protein into α -GTP and $\beta\gamma$ dimer¹⁰. Free G_{α} -GTP and/or $G_{\beta\gamma}$ then act on multiple effectors such as adenylyl cyclases, phospholipases, receptor kinases and ion channels¹⁰⁻¹². Exogenously applied G_{α} ^{4,5} or $G_{\beta\gamma}$ ^{6,7} have been reported to activate the muscarinic potassium channel from the heart, $I_{K(ACh)}$, and there has been debate concerning the precise role of these G protein subunits. Although the discrepancies between the different observed effects have been suggested to be partly due to contaminants¹³ or detergent^{14,15} applied together with the purified G protein subunits to the atrial membranes, it remains possible that different isoforms of channels account for some of the differences reported. Having cloned the rat muscarinic potassium channel, GIRK1, we used a heterologous expression system to test the effect of different G protein subunits on GIRK1 activation.

Coexpression of GIRK1 and M2 muscarinic receptor in *Xenopus* oocytes results in carbachol-induced inwardly rectifying potassium channel activity⁸. Coexpression of GIRK1 with the G protein subunits $\alpha i-2$, and $\beta 1$ and $\gamma 2$, in contrast, results in high basal activity of this inwardly rectifying potassium channel (Fig. 1a, d see also ref. 8). Subtracting the endogenous current from the total current produced by coexpressing GIRK plus $\alpha i-2$, $\beta 1$ and $\gamma 2$ reveals an inwardly rectifying current that activates slowly with hyperpolarization (Fig. 1e), typical of the muscarinic potassium channel. Previous studies have shown that the basal

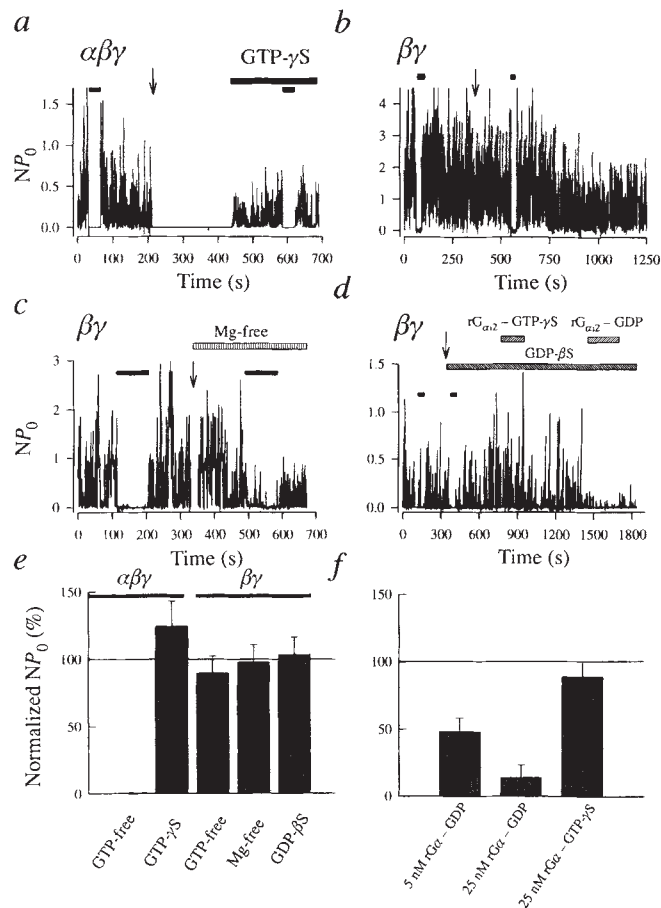
activity of the atrial muscarinic potassium channel arises from the slow turnover rate of the G proteins^{16,17}. The much larger basal activities of GIRK1 in oocytes coexpressing GIRK1 and all three G protein subunits are probably due to the elevated level of expressed G proteins; background turnover rate of G_i may be independent of its functional interaction with agonist-free receptors¹⁸. To see whether $\alpha i-2$ or $\beta 1\gamma 2$ subunits are responsible for the basal current, we coexpressed GIRK1 with either $\alpha i-2$ or $\beta 1, \gamma 2$. The coexpression of $\beta 1, \gamma 2$ with GIRK1 resulted in an inwardly rectifying current indistinguishable from that produced by $\alpha i-2, \beta 1, \gamma 2$ with GIRK1 (Fig. 1a, b, d, f). Coexpression of GIRK1 with $\alpha i-2$ (Fig. 1c, d, f) or GIRK1 alone (Fig. 1f), in contrast, resulted in a smaller inwardly rectifying current which could not be distinguished from the endogenous current (see Fig. 1 legend for details). These results suggest that $G_{\beta\gamma}$ may account for the high basal GIRK1 activity observed when all three G protein subunits are coexpressed with the channel.

To determine whether the endogenous G_{α} activity is required for the channel activities observed in oocytes which coexpress $\beta 1, \gamma 2$ and GIRK1, we excised membrane patches into cytoplasmic solutions that lacked GTP or Mg^{2+} to favour the GDP-bound form of G_{α} . GIRK1 single-channel activity persisted in cytoplasmic solutions that were GTP-free (Fig. 2b), Mg^{2+} -free (Fig. 2c), or contained GDP- β S (Fig. 2e). Therefore, conditions that support activation of G_{α} do not appear to be required for the activation of GIRK1.

Although the activities of GIRK1 channels in oocytes coexpressing $\beta 1, \gamma 2$ with GIRK1 persisted in the absence of cytoplasmic GTP, the open probability of these channels in oocytes coexpressing $\alpha i-2, \beta 1, \gamma 2$ with GIRK1 decreased to

FIG. 2 GIRK1 single-channel activity requires $G_{\beta\gamma}$ and is reduced by $G_{\alpha i-2}$ -GDP. a-d, Open probability (NPo) plotted as a function of time. a, Oocytes coexpressing GIRK1 with $\alpha i-2\beta 1\gamma 2$. Open channel probability decreased if patches are excised (arrow) into a cytoplasmic GTP-free solution ($1 \pm 1\%$ of control, $n=4$), but increased if the solution contained 100 μ M GTP- γ S ($124 \pm 19\%$; $n=2$, also see ref. 8). b-d, Oocytes coexpressing GIRK1 with $\beta 1\gamma 2$. Channel activity persisted when membrane patches were excised into cytoplasmic GTP-free solution (b, $90 \pm 13\%$, $n=13$, ANOVA followed by LSD, $P<0.005$). In addition, channel activity did not decrease in GTP-free solutions that were Mg^{2+} -free (c, $98 \pm 13\%$, $n=3$, $P>0.05$) or had 100 μ M GDP- β S (e, $103 \pm 13\%$, $n=10$, $P>0.05$) or had 500 μ M GDP ($68 \pm 31\%$, $n=2$, $P>0.05$). d, Exogenously applied 25 nM myristoylated recombinant $G_{\alpha i-2}$ -GTP- γ S (r-myr- $G_{\alpha i-2}$ -GTP- γ S) did not reduce single-channel open probability ($90 \pm 15\%$, $n=4$). In contrast, 5 and 25 nM of r-myr- $G_{\alpha i-2}$ -GDP reduced single-channel activity to $48 \pm 10\%$ ($n=6$) and $14 \pm 9\%$ ($n=3$) of control levels, respectively (the endogenous G_{α} was likely to be in the GDP- β S-bound form). The membrane potential was held at -60 mV and briefly stepped to either $+40$ or $+50$ mV (solid bars) to test for inward rectification (a-d). e, Bar graph showing the open probability (NPo) measured at least 1 min after exposure to different cytoplasmic solutions normalized to the NPo measured before excision. f, Bar graph showing the NPo measured after 3-5 min exposure to 5 or 25 nM r-myr- $G_{\alpha i-2}$ -GDP or 25 nM r-myr- $G_{\alpha i-2}$ -GTP- γ S normalized to the NPo preceding protein application.

METHODS. Recordings of single-channel activity and the composition of the recording solutions are as described previously⁸. Stock solutions of GTP- γ S (100 mM), GDP (500 mM), GDP- β S (100 mM) were diluted immediately before use. Probability of opening during 1-2 min of recording was calculated by integration method or from idealized current records (FITCHAN). The r-myr- $G_{\alpha i-2}$ fusion protein, tagged with Glu-Glu epitope (EEEEYMPME) in the C terminus of mouse $G_{\alpha i-2}$ ²⁴, was purified from crude cytosolic extracts of infected Sf9 cells as previously described²⁵. The purified protein, stored in 50 mM HEPES, 1 mM EDTA and 1 mM β -mercaptoethanol (β ME), showed GTP- γ S binding and steady-state GTPase rates similar to those previously published for r- $G_{\alpha i-2}$ ²⁶. r-myr- $G_{\alpha i-2}$ -GTP- γ S was prepared by preincubating with 20 μ M GTY- γ S and 10 mM $MgSO_4$ and its concentration determined by GTP- γ S binding. r-myr- $G_{\alpha i-2}$ -GDP (6.5 μ M) or r-myr- $G_{\alpha i-2}$ -GTP- γ S (5 μ M) was diluted with the cytoplasmic solution plus either 500 μ M GDP or 100 μ M GDP- β S before each experiment. All recombinant protein solu-



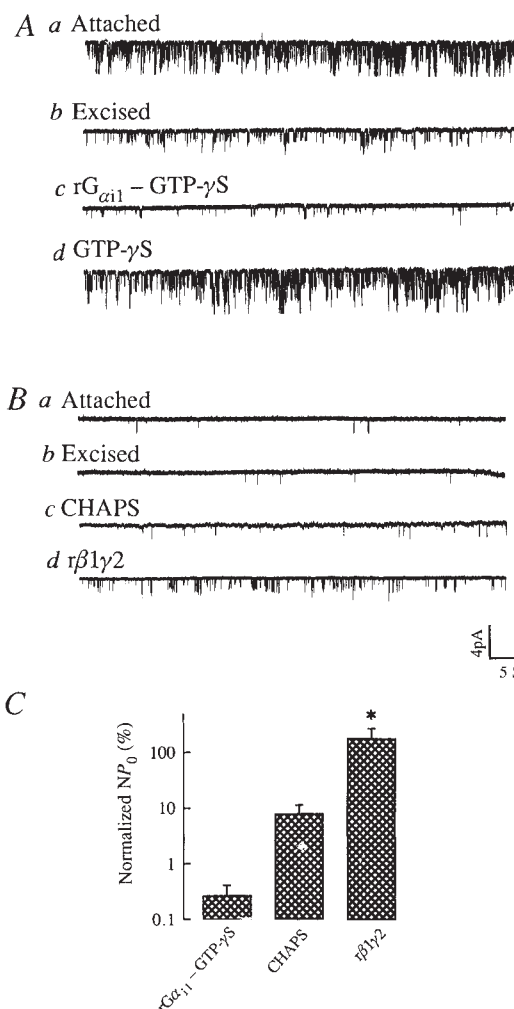
tions were supplemented with 1 mg ml⁻¹ BSA and applied to the patch through a 50-100 μ m perfusion pipette.

FIG. 3 GIRK1 single-channel activity is increased by application of recombinant $G\beta 1\gamma 2$ but not by r-myr- $G_{\alpha i-1}$ -GTP- γS . A–C, Oocytes coexpressing GIRK1 and $\alpha i-2$, $\beta 1$, $\gamma 2$. A, B, Continuous single-channel recordings at -60 mV (1 min segments separated by 1–10 min). The basal single channel activity observed in cell-attached configuration (Aa) decreased upon excision into a GTP-free solution (b). GIRK1 was not reactivated by 50 nM r-myr- $G_{\alpha i-1}$ -GTP- γS (c, 0.26 ± 0.14 fold, $n = 2$) but was reactivated by 100 μM GTP- γS (d). Cell-attached patch with low basal activity (Ba) showed similar level of channel activity after excision into GTP-free solution (b). Application of vehicle control containing 22.7 μM CHAPS had no significant effect on GIRK1 activity (c, 7.8 ± 3.5 fold, $n = 8$; $P > 0.05$ by paired Student's *t*-test (CHAPS versus GTP-free)) whereas application of 50 nM r $G\beta 1\gamma 2$ increased GIRK1 single-channel activity (d, 178 ± 95 fold; $n = 8$, asterisk indicates $P < 0.05$ by paired Student's *t*-test ($\beta\gamma$ versus GTP-free)). C, Bar graph showing NPo measured 3–5 min after exposure to the indicated solution normalized to the NPo in GTP-free solution.

METHODS. Myristoylated recombinant $G_{\alpha i-1}$ (r-myr- $G_{\alpha i-1}$) was produced by coexpression of the α -subunit with *N*-myristoyltransferase in *Escherichia coli* and purified as previously described²⁷. r-myr- $G_{\alpha i-1}$ was activated with GTP- γS , separated from free nucleotide by gel filtration and used within 72 h of activation. The same sample could inhibit $G_{\alpha s}$ -stimulated type VI adenylyl cyclase²⁸. For r $G\beta 1\gamma 2$, Sf9 cells were co-infected with baculoviruses encoding $\beta 1$, $\gamma 2$ and a modified form of $G_{\alpha i-1}$ containing an internal hexa-histidine sequence. Membranes were prepared and extracted with Na-cholate as described²⁹. The extract was diluted fourfold in 20 mM Na-HEPES (pH 8.0), 400 mM NaCl, 0.5% polyoxyethylene 10 lauryl ether ($C_{12}E_{10}$), 10 μM GDP, 10 mM β ME, 1 mM $MgCl_2$ and loaded onto a Ni-NTA (Qiagen). r $G\beta 1\gamma 2$ was absorbed at 15 °C with buffer supplemented with 100 mM $MgCl_2$, 10 mM NaF, 30 μM $AlCl_3$ and with $C_{12}E_{10}$ replaced by 1% Na-cholate, ultrafiltered and diluted with 20 mM Tris-Cl (pH 8.0), 1 mM Na-EDTA, 2 mM DTT, 11.38 mM CHAPS (0.7%). The material was further purified on a MONO-Q HR 5/5 ion exchange column (0–250 mM NaCl gradient in the same CHAPS buffer). Purified r $G\beta 1\gamma 2$ was concentrated and stored in 20 mM Na-HEPES, 50 mM NaCl, 3 mM DTT, 11.38 mM CHAPS and diluted into cytoplasmic solution containing 100–500 μM GDP and 1 mg ml⁻¹ BSA.

~1% of control when the membrane patch was excised to a GTP-free solution (Fig. 2a, e). These channels can be reactivated by 50 nM recombinant $G\beta 1\gamma 2$ (r $G\beta 1\gamma 2$) or 100 μM GTP- γS , but not by the detergent solution used as a vehicle for $G_{\beta\gamma}$ (Figs 2a, e and 3), similar to what has been shown for the atrial muscarinic potassium channel^{6,7,15,19}. By contrast, 50 nM of myristoylated recombinant $G_{\alpha i-1}$ -GTP- γS (r-myr- $G_{\alpha i-1}$ -GTP- γS), which could inhibit $G_{\alpha s}$ -stimulated type VI adenylyl cyclase (not shown), did not activate GIRK1 (Fig. 3). Thus, $G_{\beta\gamma}$ but not G_{α} -GTP- γS appears to activate the muscarinic potassium channel. Exposing the cytoplasmic side of membrane patches from oocytes coexpressing $\beta 1$, $\gamma 2$ and GIRK1 to exogenously applied myristoylated recombinant $G_{\alpha i-2}$ -GDP (r-myr- $G_{\alpha i-2}$ -GDP) reduced channel open probability, whereas exposing the patch to r-myr- $G_{\alpha i-2}$ -GTP- γS , which has lower affinity for $G_{\beta\gamma}$, did not reduce the single-channel open probability (Fig. 2d, f). These results are consistent with the idea that G_{α} blocks the activation of GIRK1 in GTP-free cytoplasmic solution by hydrolysing GTP to form G_{α} -GDP, which then binds $G_{\beta\gamma}$ and reduces GIRK1 channel activity, as has been suggested for the action of G_{α} on $I_{K(ACh)}$ ¹⁵.

If GIRK1 channel is activated by $G_{\beta\gamma}$, other proteins that interact with $G_{\beta\gamma}$ might affect channel activation. The β -adrenergic receptor kinase 1 (β ARK1) interacts with $G_{\beta\gamma}$ and is thereby targeted to its membrane-bound substrate²⁰; the binding site for $G_{\beta\gamma}$ has been localized to the C-terminal domain of β ARK1²¹. We have found a limited level of sequence similarity between the C-terminal domain of GIRK1 and that of β ARK1 (Fig. 4a), suggesting that GIRK1 might interact with $G_{\beta\gamma}$ in a manner analogous to β ARK1. To test whether a C-ter-



минаl fragment of β ARK1 could sequester $G_{\beta\gamma}$ and reduce channel activity, we exposed the cytoplasmic side of membrane patches from oocytes expressing $\beta 1$, $\gamma 2$ and GIRK1 to a fusion protein containing the C-terminal fragment of β ARK1 (His₆- β ARK1). This treatment reduced channel open probability. As control, we tested the effect of a C-terminal fragment of rhodopsin kinase (His₆-RK, Fig. 4a) which is homologous to β ARK1 but does not interact with $G_{\beta\gamma}$ ²⁰. This control fusion protein had no effect on GIRK1 single-channel open probability (Fig. 4b). Given that the C-terminal fragment of β ARK1 is not known to have any enzymatic activities or other functions besides binding $\beta\gamma$, the inhibition of channel activity by His₆- β ARK1 provides further support for the role of $G_{\beta\gamma}$ in the activation of GIRK1.

Whether the C-terminal hydrophilic domain of GIRK1 is involved in channel activation by $G_{\beta\gamma}$ remains to be determined in the future. Truncating GIRK1 in the C-terminal hydrophilic domain at proline 462 resulted in functional expression in oocytes that also coexpress $\beta 1$, $\gamma 2$ but not in oocytes injected with this truncated mutant alone, indicating that the last 39 residues are not essential for $G_{\beta\gamma}$ activation. Further deletions (from the C terminus up to leucine 403 or proline 355) abolished functional expression even when these truncation mutants were coexpressed with $\beta 1$, $\gamma 2$. Although these results are compatible with the hypothesis that the C-terminal hydrophilic domain is important for channel activation, we cannot rule out the possibility that the large deletions affect the formation or stability of the mutant channels.

In summary, the cloned muscarinic potassium channel expressed in *Xenopus* oocytes can be activated by $G_{\beta\gamma}$ but not

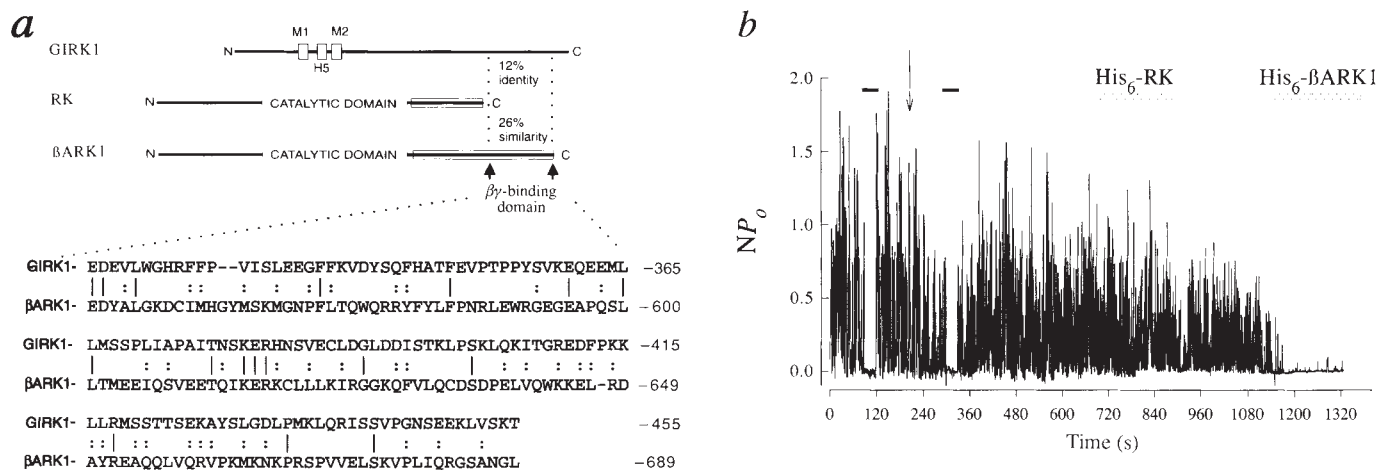
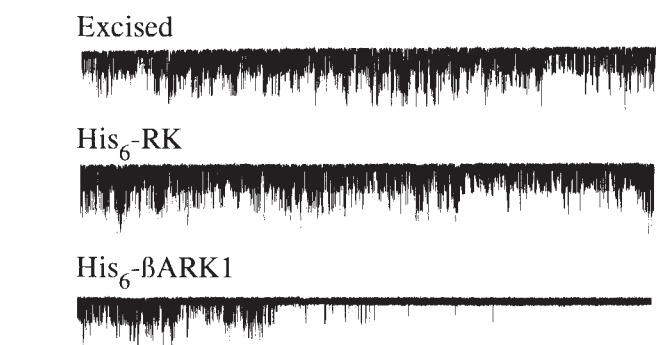


FIG. 4 The GIRK1 channel activation in oocytes coexpressing $\beta 1\gamma 2$ is reduced by bacterially expressed C-terminal fragment of β -adrenergic receptor kinase 1 (β ARK1) but not by rhodopsin kinase (RK). **a**, Schematic representation of GIRK1, RK and β ARK1 proteins and the C-terminal fragments contained in the fusion protein His 6-RK and His 6- β ARK1 (boxes) (upper part) and an alignment of the C-terminal domain of GIRK1 and the $G_{\beta\gamma}$ binding domain of β ARK1²¹ over a region of 140 amino acids (lower part). (I, identical; :, conserved substitution). **b**, GIRK1 single-channel activities (at -60 mV) were reduced by 10–20 μ M His 6- β ARK1 to $13 \pm 6\%$ of control ($n=6$). However, channel activities were not reduced by 10–20 μ M of His 6-RK ($101 \pm 7\%$, $n=4$). The channel activity in the presence of His 6- β ARK1 was significantly different from that in the presence of His 6-RK (ANOVA, $P<0.001$). The inward rectification of GIRK1 was evident from the absence of current on depolarization to +40 mV (solid bar) before or after excising the membrane patch (arrow). NP₀ plot of the continuous recording is shown above the 3-min segments of recording.

METHODS. The His 6-RK and the His 6- β ARK1, containing the terminal 91 amino acids of RK and 222 amino acids of β ARK1, respectively, were prepared as described previously³⁰. Fractions containing the pro-



tein of interest were dialysed against 50 mM HEPES, 100 mM KCl, 0.5 mM EDTA, 1 mM β ME and 10% glycerol. The dialysis buffer had no apparent effect on channel activity. Protein concentrations were determined using the Bradford assay (Biorad) with BSA as a standard.

by r-myr- $G_{\alpha i-1}$ -GTP- γ S. Activation of GIRK1 by $G_{\beta\gamma}$ may be terminated by G_{α} -GDP which binds $G_{\beta\gamma}$. These studies represent the first step in the analysis of the mechanism of channel activation by G protein. The muscarinic potassium channel in turn may accelerate the rate of GTP hydrolysis of the G_{α} , because its closing rate following the termination of muscarinic receptor activation far exceeds the rate of hydrolysis of purified G_{α} ^{22,23}. It will be interesting to examine the interactions between the muscarinic potassium channel and the different G protein subunits and the effects of these interactions on the function of the G protein as well as the channel. □

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A tyrosyl-tRNA synthetase can function similarly to an RNA structure in the *Tetrahymena* ribozyme

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GROUP I introns are highly structured RNAs which catalyse their own splicing by guanosine-initiated transesterification reactions^{1,2}. Their catalytic core is generally stabilized by RNA-RNA interactions within the core and with peripheral RNA structures^{3,4}. Additionally, some group I introns require proteins for efficient splicing *in vivo*⁵. The *Neurospora* CYT-18 protein, the mitochondrial tyrosyl-transfer RNA synthetase (mt TyrRS), promotes splicing of the *Neurospora* mitochondrial large ribosomal RNA (LSU)

and other group I introns by stabilizing the catalytically active structure of the intron core⁶⁻⁸. We report here that CYT-18 functions similarly to a peripheral RNA structure, P5abc, that stabilizes the catalytic core of the *Tetrahymena* LSU intron. The CYT-18 protein and P5abc RNA bind to overlapping sites in the intron core, inducing similar conformational changes correlated with splicing activity. Our results show that a protein can play the role of an RNA structure in a catalytic RNA, a substitution postulated for the evolution of nuclear pre-messenger RNA introns from self-splicing introns^{9,10}.

The CYT-18 protein bound strongly to most group I introns surveyed, but not to the *Tetrahymena* LSU intron⁸. A possible explanation was that the *Tetrahymena* intron contains a large, peripheral extension of the P5 stem, P5abc, found in some subgroup IB and IC introns (Fig. 1a)³. P5abc interacts with P4-P6-P6a, the same region of the catalytic core bound by CYT-18^{8,11,12}. To investigate whether P5abc impedes CYT-18 binding, we used a nitrocellulose filter binding assay to compare the interaction of CYT-18 with the wild-type *Tetrahymena* intron and a derivative lacking P5abc (Δ P5abc intron)¹³ (Fig. 1b, c). The wild-type *Tetrahymena* intron showed only nonspecific binding at higher protein concentrations (dissociation constant, $K_d > 300$ nM), whereas the Δ P5abc intron bound CYT-18 similarly to the *Neurospora* mt LSU intron, at a stoichiometry of

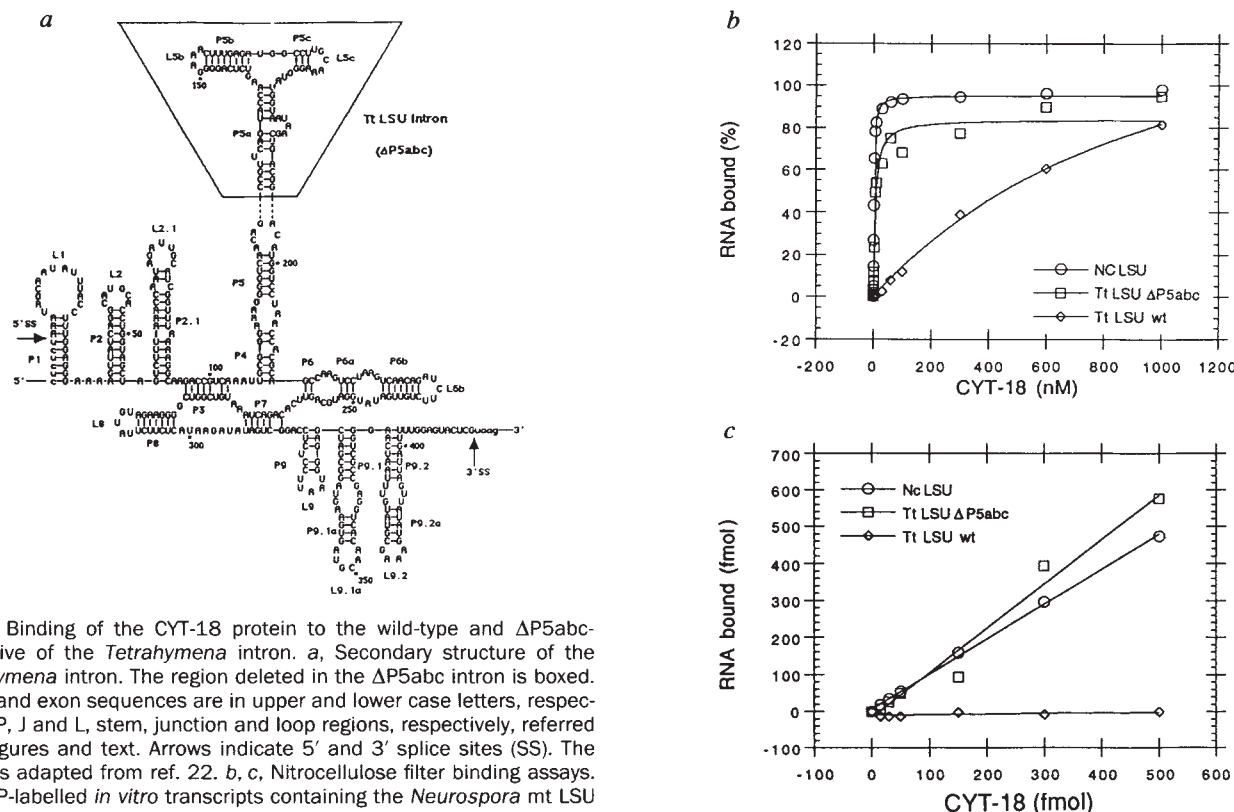


FIG. 1 Binding of the CYT-18 protein to the wild-type and Δ P5abc-derivative of the *Tetrahymena* intron. *a*, Secondary structure of the *Tetrahymena* intron. The region deleted in the Δ P5abc intron is boxed. Intron and exon sequences are in upper and lower case letters, respectively. P, J and L, stem, junction and loop regions, respectively, referred to in figures and text. Arrows indicate 5' and 3' splice sites (SS). The figure is adapted from ref. 22. *b*, *c*, Nitrocellulose filter binding assays. ³²P-UTP-labelled *in vitro* transcripts containing the *Neurospora* mt LSU intron (2.2 nM), the *Tetrahymena* intron (10 nM) or the Δ P5abc intron (10 nM) were incubated with increasing concentrations of CYT-18 protein, and binding assayed by retention of the ³²P-labelled RNA on a nitrocellulose filter⁸. *b*, Per cent RNA bound versus concentration of CYT-18 dimer. *c*, Low CYT-18 protein concentrations are replotted to show the amount of RNA retained against the amount of CYT-18 dimer. METHODS. The *Neurospora* mt LSU intron was transcribed by phage T3 RNA polymerase from plasmid pBD5A linearized with *Ban*I⁸. The wild-type and Δ P5abc *Tetrahymena* introns were transcribed by phage T7 RNA polymerase from *Eco*RI-linearized plasmids pT7T1A3²³ and pT7T1A3 Δ P5abc¹³, respectively. *Neurospora* mt LSU intron transcription was in the presence of 100 μ Ci [α -³²P]UTP (3,000 Ci mmol⁻¹) plus

250 μ M of each unlabelled NTP. Transcription of the *Tetrahymena* introns was with higher NTP concentrations (1 mM), which suppress self-splicing by binding free Mg²⁺ (ref. 24). DNase I-treated transcripts were purified through a Sephadex G-50 spun column and refolded by incubation in TMK (10 mM Tris-HCl, pH 7.5, 5 mM MgCl₂, 100 mM KCl) for 10 min at 55 °C, followed by 10 min at room temperature. CYT-18 protein was synthesized in *Escherichia coli* from expression plasmid pEX560 (previously designated pEX550A²⁵) and purified by polyethyl-eneimine (PEI) precipitation, (NH₄)₂SO₄ fractionation and CM-Sepharose chromatography (R. Saldanha *et al.*, manuscript in preparation). For binding assays, ³²P-UTP-labelled RNA and CYT-18 protein were mixed in 500 μ l of TMK, 10% glycerol, 0.05 mg ml⁻¹ bovine serum albumin, incubated at room temperature for 30 min, and filtered through nitrocellulose⁸.

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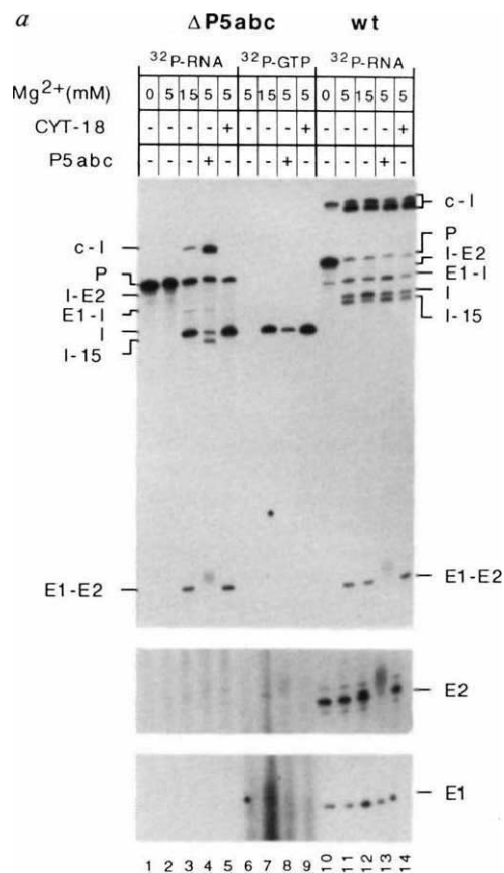
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FIG. 2 The CYT-18 protein splices the Δ P5abc derivative of the *Tetrahymena* intron. **a**, Splicing reactions were with the Δ P5abc intron or the wild-type *Tetrahymena* intron with or without 200 nM CYT-18 protein or 1 μ M P5abc RNA. 32 P-RNA indicates reactions with 32 P-UTP-labelled precursor plus unlabelled GTP, and 32 P-GTP indicates reactions with unlabelled precursor plus 32 P-GTP. Lanes 1, 10: precursor before incubation; lanes 2, 6, 11: control reactions in 5 mM $MgCl_2$; lanes 3, 7, 12: reactions in 15 mM $MgCl_2$ and 2 mM spermidine; lanes 4, 8, 13: reactions in 5 mM $MgCl_2$ with 1 μ M P5abc RNA; lanes 5, 9, 14: reactions in 5 mM $MgCl_2$ with 200 nM CYT-18 protein. Sections showing E1 and E2 (bottom) are from a longer exposure of the autoradiogram. In lanes 4 and 13, the presence of P5abc RNA distorts the migration of smaller RNAs containing the 3' exon, possibly because of base pairing between complementary sequences in the polylinker regions of these RNAs. The wild-type *Tetrahymena* intron produces both a 399- and 413-nt c-I²⁶. c-I, Circular intron; E1, 5' exon; E2, 3' exon; E1-E2, ligated exons; I, linear intron; I-15, intron with 15 nt cleaved from its 5' end; P, precursor RNA. **b**, Kinetic parameters. The rate of splicing was determined at six or more GTP concentrations, ranging from 0.1 μ M to 1 mM. The increase in the amount of ligated exons was plotted as a function of time, and k_{obs} was determined from the linear part of the graph. k_{cat}/K_m was determined from plots of k_{obs} as a function of GTP concentration²⁷. K_m was determined from Hanes-Woolfe plots of the amount of ligated exons as a function of GTP concentration. k_{cat} was calculated from the k_{cat}/K_m and K_m values.

METHODS. Precursor RNAs were purified by electrophoresis in 4% polyacrylamide, 7 M urea gels. After extraction with phenol-CIA (phenol-chloroform-isoamyl alcohol, 25:24:1), desalting through Sephadex G-50, and ethanol precipitation, precursor RNAs were refolded in splicing medium without GTP for 10 min at 55 °C, followed by 5 min at 37 °C. P5abc RNA was transcribed by T7 RNA polymerase from pP5abcX linearized with XbaI¹⁴. In **a**, splicing reactions were with 54 nM 32 P-UTP-labelled precursor RNA (50 nCi μ mol⁻¹) plus 250 μ M GTP or with 58 nM unlabelled precursor RNA plus 20.3 μ Ci [α - 32 P]GTP (250 Ci mmol⁻¹ in 20 μ l of 100 mM KCl, 20 mM Tris-HCl, pH 7.5, 5 mM dithiothreitol, and either 5 mM $MgCl_2$ or 15 mM $MgCl_2$ plus 2 mM spermidine. The reactions were for 30 min at 37 °C. In **b**, splicing reactions were at 37 °C with 32 P-UTP-labelled precursor RNA (45 nM, 95 nCi μ mol⁻¹) plus 200 nM CYT-18 or 1 μ M P5abc RNA in 220 μ l of the reaction medium containing 5 mM Mg^{2+} . Splicing products were analysed in 5% polyacrylamide, 7 M urea gels and quantified with a betascope 603 (Betagen). Other methods were as for Fig. 1.

one RNA molecule per CYT-18 protein dimer. As discussed elsewhere, stoichiometric titration measurements provide only an upper limit of the strength of CYT-18 binding to group I introns (R. Saldanha *et al.*, manuscript in preparation). The K_d values for the binding of CYT-18 to the *Neurospora* mt LSU intron and the Δ P5abc intron, calculated from measurements of k_{off} and k_{on} , rate constants for the dissociation and formation of CYT-18/intron RNA complexes, were <3 pM and 6 pM, respectively (R. Saldanha *et al.*, manuscript in preparation; and data not shown). The binding of CYT-18 to the Δ P5abc intron appears to be substantially stronger than is binding of P5abc RNA, as estimated from published gel shift assays (>250 nM)¹⁴ and from our measurements of K_m^{P5abc} , the Michaelis constant for P5abc RNA (214 nM in splicing reactions with 10 nM 32 P-labelled Δ P5abc precursor RNA and saturating GTP).

Confirming previous results¹⁴, Fig. 2a shows that the Δ P5abc intron does not self-splice in reaction medium containing 5 mM Mg^{2+} (lanes 2, 6), but splicing is restored under high Mg^{2+} conditions (lanes 3, 7) or when the P5abc RNA is supplied in *trans* as a separate RNA molecule (lanes 4, 8). Strikingly, the CYT-18 protein replaced P5abc RNA to restore the splicing of the Δ P5abc intron at low Mg^{2+} concentrations (lanes 5, 9). The CYT-18-assisted reaction produced ligated exons and linear intron, but unlike other reactions gave only traces of circular intron (c-I) and little if any I-15 product, resulting from circle reopening. Controls showed that splicing was not restored by boiled CYT-18 (not shown) and that neither P5abc RNA nor CYT-18 affected the splicing products of the wild-type *Tetrahymena*



mena intron (Fig. 2a, lanes 13, 14). The catalytic rate constant k_{cat} and the Michaelis constant for GTP K_m^{GTP} values for the P5abc-assisted splicing of the Δ P5abc intron were close to those for the wild-type intron, whereas CYT-18-assisted splicing had a 20-fold lower k_{cat} and a 13-fold higher K_m^{GTP} (Fig. 2b). Controls showed no effect of CYT-18 on the rate of splicing or K_m^{GTP} of the wild-type intron (not shown). Thus, we conclude that CYT-18 only partially compensates for the loss of the P5abc structure and most probably induces a suboptimal structure of the catalytic core. The lack of circular intron could be accounted for simply by a suboptimal splicing structure¹⁵, but could also reflect that the CYT-18-induced structure discriminates against cryptic splice sites or that CYT-18 directly impedes structural changes required for cyclization¹⁶.

The effect of CYT-18 on the structure of the Δ P5abc intron was investigated directly by chemical structure mapping. CYT-18/intron RNP complexes are stable in dimethyl sulphate (DMS) or diethylpyrocarbonate (DEPC), but not in Fe(II)-EDTA. Previous DMS- and DEPC-structure mapping of the wild-type *Tetrahymena* intron at 10 mM Mg^{2+} showed that protected bases occur both in stem regions and in single-stranded regions protected by tertiary structure (such as L2.1, J3/4, J4/5, J5/4, J6/6a, J7/3, J8/7, L9 and L9.1a)¹⁷⁻¹⁹. We observed substantially the same protection pattern for the wild-type intron at 5 mM Mg^{2+} (L-21 form; data not shown) and for the Δ P5abc intron under high Mg^{2+} conditions (Fig. 3a, lanes 4, 8, 13, 17; Fig. 3b, middle). By contrast, the Δ P5abc RNA at 5 mM Mg^{2+} showed only a subset of protected bases, corresponding pre-

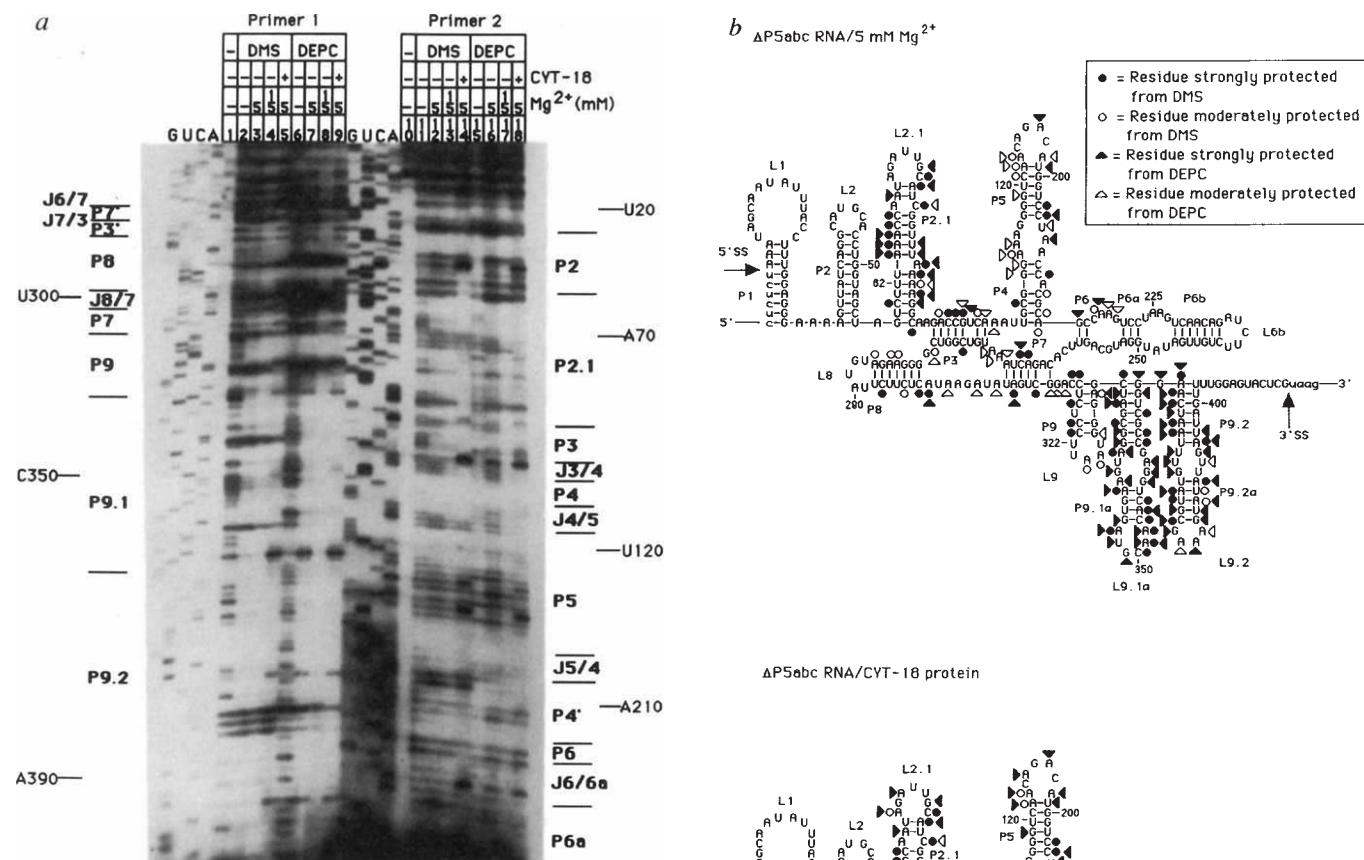


FIG. 3 Chemical modification of the *Tetrahymena* $\Delta P5abc$ intron. **a**, $\Delta P5abc$ RNA was incubated with DMS, which modifies N1 of adenines and N3 of cytosines, and DEPC, which primarily modifies N7 of adenines, but also other bases²⁸. Modified nucleotides were detected as blocks to primer extension one base before the modified nucleotide. Lanes 1, 10: $\Delta P5abc$ RNA (starting material); lanes 2, 6, 11, 15: modification at 55 °C in 20 mM HEPES/KOH, pH 7.5 (denaturing); lanes 3, 7, 12, 16: modification at 37 °C in buffer plus 100 mM KCl, 5 mM $MgCl_2$; lanes 4, 8, 13, 17: modification at 37 °C in buffer plus 100 mM KCl, 15 mM $MgCl_2$, 2 mM spermidine; lanes 5, 9, 14, 18: modification at 37 °C in buffer plus 100 mM KCl, 5 mM $MgCl_2$, 60 nM CYT-18. Nucleotide positions and regions of the *Tetrahymena* intron are indicated. **b**, Residues protected in the $\Delta P5abc$ intron. Circles and triangles indicate residues modified under denaturing conditions, but protected from DMS and DEPC, respectively, under the conditions shown. Filled and open symbols indicate strong (>50%) and moderate (20–50%) protections, respectively. Residues strongly protected in the presence of CYT-18, but not in 15 mM $MgCl_2$, 2 mM spermidine: A72, A74, U287, G288, A301, A302 for DEPC, and A66, C81, A87, A89, A90, C99, G100, A103, A104, A105, A113, A214, A304, A306, A324, A325 for DMS. **METHODS**. $\Delta P5abc$ RNA (20 nM pT7T1A3 $\Delta P5abc$ /EcoRI) was preincubated in 100 μ l reaction medium for 10 min at 37 °C, then modified with 200 mM DMS or 800 mM DEPC for 30 s at 37 °C or 55 °C (denaturing conditions). Reactions were terminated by adding 4 mM EDTA plus 10 μ g *E. coli* tRNA, followed by phenol-CIA extraction and ethanol precipitation. Modified RNAs were reverse transcribed with M-MLV reverse transcriptase (GIBCO BRL), from 5' end-labelled primer 1 (5'-AGGCATTGGCTACCTTA) or primer 2 (5'-CTGCATCCATATCAACAGAA-GATCTGTGTA). Products were separated in a 6% polyacrylamide, 7 M urea gel, and quantified by densitometry of shorter autoradiogram exposures. Levels of modification were assigned after normalizing the amount of primer extension products in each lane. Dideoxy sequencing reactions obtained with Sequenase (USB) from plasmid pT7T1A3 $\Delta P5abc$ using the same primers were run in parallel lanes. Bases A2 to U59 and U221 to U259 were not adequately resolved for analysis. Less than 5% of the precursor RNA spliced during transcription (lanes 1, 10), and no hydrolysis of the precursor occurred during the experiment, ensuring that the entire population of precursor RNAs was monitored (not shown).

FIG. 4 Lead-induced cleavage of the wild-type and Δ P5abc *Tetrahymena* introns. 32 P-UTP-labelled precursor RNAs containing the *Tetrahymena* intron or the Δ P5abc intron were incubated in the presence or absence of P5abc RNA or CYT-18 protein in reaction media containing Pb acetate and/or $MgCl_2$, as indicated. The sizes of the 5' and 3' fragments (indicated) are those expected for a single Pb^{2+} cleavage in the J8/7 region. Primer extension mapping confirmed that Pb^{2+} cleavage of the Δ P5abc intron in the presence of CYT-18 occurred 3' of A304 (not shown), as in the wild-type *Tetrahymena* intron²⁰. In lane 6, the 3' cleavage product was not detected, presumably because of its interaction with the P5abc RNA (see Fig. 2). M, RNA size markers; 5' and 3', 5'- and 3'- cleavage products, respectively.

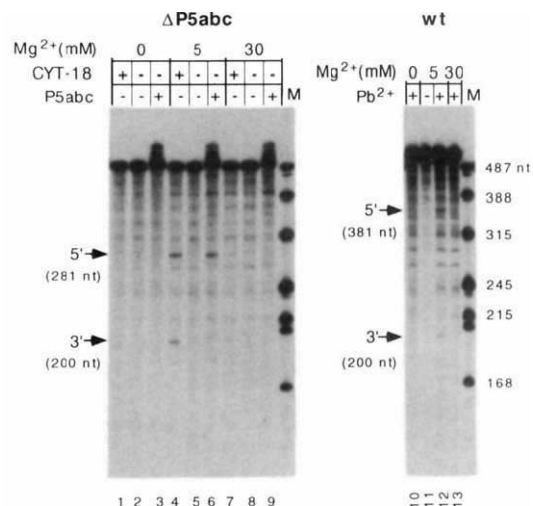
METHODS. Reactions were with 32 P-UTP-labelled precursor RNAs containing the wild-type or Δ P5abc *Tetrahymena* introns (pT7T1A3/*Eco*RI or pT7T1A3 Δ P5abc/*Eco*RI, respectively; 10 mM, 80 nCi μ mol⁻¹; prepared as in Fig. 1), in the presence or absence of 1 μ M P5abc RNA or 200 nM CYT-18 protein. RNAs were incubated for 5 min at 37 °C in 20 μ l of solution containing 100 mM KCl, 20 mM Tris-HCl, pH 7.5, 5 mM DTT, 20 units RNase inhibitor (USB) plus the concentrations of $MgCl_2$ indicated. The samples were transferred to room temperature, lead acetate was added to 20 mM, and the incubation was continued for 3 min. The reactions were terminated by adding EDTA to 50 mM and extracting with phenol-CIA. RNAs were analysed by electrophoresis in a 5% polyacrylamide, 8 M urea gel, followed by autoradiography.

dominantly to bases in stem regions, whereas many previously protected bases in loop or junction regions showed increased modification (such as J3/4, J4/5, J5/4, J6/6a, J7/3, J8/7, L9; Fig. 3a, lanes 3, 7, 12, 16; Fig. 3b, top). These results indicate that much of the conserved secondary structure can form in the absence of P5abc, but that P5abc is required for formation of tertiary structure at low Mg^{2+} concentration.

Notably, the binding of CYT-18 to the Δ P5abc intron at low Mg^{2+} concentration increased the protection of key bases in single-stranded regions, as expected for the restoration of tertiary structure required for splicing activity (see J3/4, J4/5, J5/4, J6/6a, J7/3, J8/7, L9; Fig. 3a, lanes 5, 9, 14, 18; Fig. 3b, bottom). CYT-18 did not, however, fully restore the wild-type tertiary structure, as judged by the modification of a number of bases that were protected in the Δ P5abc intron under high Mg^{2+} conditions (23/140 and 24/124 for DMS and DEPC, respectively; see Fig. 3b) or in the wild-type *Tetrahymena* intron¹⁹. In addition, some modifications were enhanced by CYT-18, and 22 bases showed substantially increased protection in the presence of CYT-18 compared with high Mg^{2+} concentration (see legend to Fig. 3). The latter protection could reflect either the altered RNA structure or direct protection by the protein.

Confirmation that both CYT-18 and P5abc RNA induce a key component of the active tertiary structure was obtained by monitoring Pb^{2+} -induced cleavage of the intron RNA. The *Tetrahymena* and other group I introns are specifically cleaved by Pb^{2+} at two sites in the core, J8/7 (A304) and the bulged nucleotide in P7 (A263)²⁰. These Pb^{2+} -cleavages occurred only in the presence of sufficient Mg^{2+} to stabilize RNA structure, but were not observed at high Mg^{2+} concentration, which presumably competes for the metal-ion binding site²⁰. As shown in Fig. 4, we readily detected Pb^{2+} -cleavage in J8/7 in the wild-type *Tetrahymena* intron at 5 mM Mg^{2+} , but no cleavage at the bulged nucleotide in P7, which is also more difficult to detect in other introns²⁰. By contrast, the Pb^{2+} -cleavage in J8/7 was not observed in the Δ P5abc intron at 5 mM Mg^{2+} (Fig. 4, lane 5), suggesting that formation of the metal-ion-binding site is dependent on tertiary structure induced by P5abc. Notably, the Pb^{2+} -cleavage was restored by incubating the Δ P5abc intron with either P5abc RNA (Fig. 4, lane 6) or CYT-18 protein (lane 4), consistent with the restoration of active tertiary structures. As expected, the Pb^{2+} -cleavages induced by CYT-18 or P5abc were not observed in the absence of Mg^{2+} or in 30 mM Mg^{2+} (Fig. 4, lanes 1, 3, 7, 9).

Together, our results show that the CYT-18 protein can function similarly to the P5abc RNA in promoting splicing of the *Tetrahymena* intron at low Mg^{2+} . Both CYT-18 and P5abc bind



to the P4-P6-P6a region of the intron core and induce structural changes correlated with catalytic activity. Not surprisingly, CYT-18 only partially compensated for the loss of P5abc, which coevolved with the *Tetrahymena* intron. We speculate that P5abc and other peripheral extensions were acquired by preexisting group I introns as random insertions and were maintained because they conferred some selective advantage, such as increased stability or protection of the core from ribonucleases. This initial role may have favoured interactions with the core that replaced other types of structural stabilization, eventually resulting in dependence on the structure for RNA splicing. Similarly, the CYT-18-dependent group I introns in *Neurospora* mitochondria were presumably self-splicing initially and became protein-dependent as a result of modifications that impaired self-splicing^{5,21}. The discovery that CYT-18 can substitute for P5abc provides an opportunity to investigate how an RNA-protein interaction can replace an RNA-RNA interaction to promote splicing. □

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Phosphorylation and activation of the Jak-3 Janus kinase in response to interleukin-2

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INTERLEUKIN-2 is an autocrine growth factor for T cells^{1,2} which also activates other cells including B cells³ and natural killer cells⁴. The subunits of the interleukin-2 receptor (IL-2R) lack intrinsic enzymatic activity, but protein tyrosine phosphorylation is a critical event following ligand binding and *src* family kinases, such as Lck, are known to be activated by IL-2 (refs 5–9). However, IL-2 signalling can occur in the absence of receptor interaction with Lck, suggesting that other protein tyrosine kinases might be important¹⁰. Here we report that a new member of the Janus family of kinases (Jak-3) is coupled to the IL-2R in human peripheral blood T cells and natural killer cells.

Jak-3 (previously termed L-JAK) is a unique kinase of relative molecular mass 125,000 (M_r 125K) which we have cloned from natural killer (NK) cells¹¹. It differs in expression from other Jak-3s as measured both by northern analysis¹¹ and western blotting using a Jak-3 peptide antiserum which does not crossreact with other Jak-3s (Fig. 1). Expression was detected in NK and T cells but not in other cells examined, whereas the other Jak-3s were more widely expressed (data not shown). Additionally, Jak-3 was expressed at low levels in resting peripheral blood T cells, but the abundance was greatly increased after activation by either phytohaemagglutinin (PHA) (Fig. 1a, b) or anti-CD3 (data not shown), the maximal expression being seen at 24–48 h. Although Jak-3 can be induced in other cells, such as monocytes, given the appropriate stimulus (T. Musso *et al.*, manuscript in

preparation), Jak-3 induction on T-cell activation suggested that this kinase may be functionally important in these cells.

Because the Jak-3s have been shown to be important in haematopoietic receptor family signalling^{12–14} and because the induction of the IL-2 and the IL-2R α -chain are critical events in the activation of T cells^{15,16}, we next examined whether Jak-3 might be coupled to this receptor. We observed little basal tyrosine phosphorylation of Jak-3 in YT, NK 3.3, NK and HUT-78 cells (Fig. 2a, lanes 1, 3, 5; b, lane 1). However, after IL-2 treatment, marked phosphorylation was evident (Fig. 2a, lanes 2, 4, 6; b, lanes 2, 3, 5) but was not detectable in control immunoprecipitates (Fig. 2b, lane 4).

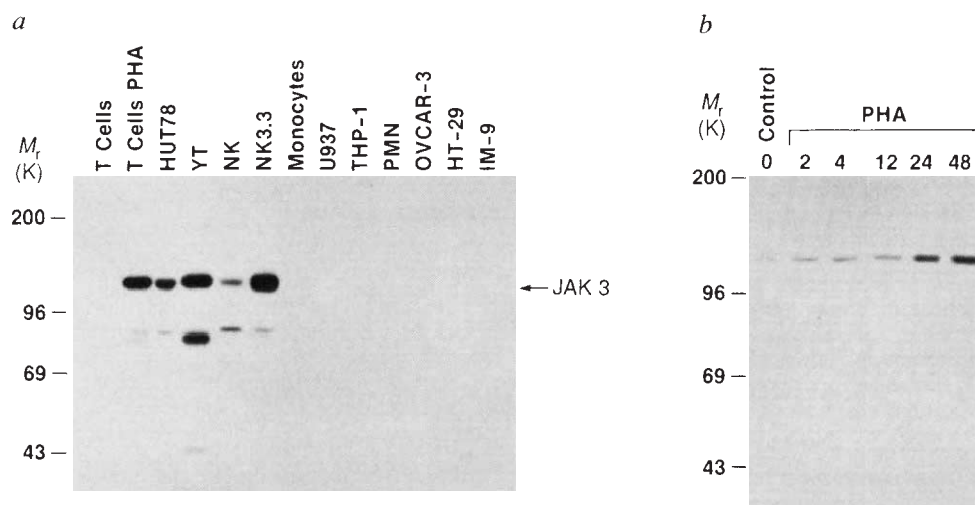
Cytokine stimulation of cells results in increased phosphotransferase activity of the Jak family kinases¹⁷. Jak-3 immunoprecipitated from YT cells (Fig. 2c, lanes 3 and 4), NK cells (lane 7) and Hut 78 cells (lane 9) stimulated with IL-2 also displayed elevated enzymatic activity, as measured by *in vitro* autophosphorylation, which peaked at about 10 min (Fig. 2c, lane 3). The kinase activity was not precipitated in the presence of competing Jak-3 peptide (Fig. 2c, lanes 2 and 4). This was exclusively tyrosyl phosphorylation, as determined by resistance to potassium hydroxide and by phosphoamino acid analysis (data not shown). Although this measure of activity may not necessarily reflect activity towards other substrates, it suggests enzymatic activation of Jak-3 by IL-2.

To link more firmly Jak-3 to the IL-2R signalling pathway, we next sought to determine if Jak-3 became associated with the IL-2R complex after ligand binding. Although Jak-3 was minimally, but detectably, associated with IL-2R β in untreated YT cells (Fig. 2d, lane 1), 15 and 30 min after IL-2 stimulation, it was readily apparent in the receptor complex (lanes 2, 3), but not in control immunoprecipitates (lane 4). Thus, although Jak-3 kinase activity was reduced by 30 min, it remains in the receptor complex; the significance of this is unclear.

IL-2 stimulation induces tyrosyl phosphorylation of a variety of substrates^{7,18} and in YT cells the most prominent substrate was a 125K polypeptide (Fig. 3a, lane 2) which comigrated with Jak-3. To determine the identity of this substrate, lysates from IL-2-treated cells were first precleared with rabbit polyclonal antisera (Fig. 3a, lanes 1 and 2), anti-Jak-3 (lanes 3 and 4) or anti-Jak-1 (lanes 5 and 6), before immunoprecipitation with antiphosphotyrosine. Depletion using anti-Jak-3 antiserum specifically removed the 125K tyrosine-phosphorylated protein, indicating that Jak-3 was one of the most prominent phosphoproteins detected in response to IL-2 in these cells (Fig. 3a).

FIG. 1 Expression of Jak-3 in activated T cells and NK cells. a, Whole-cell lysates from human peripheral blood T cells (unstimulated or stimulated for 48 h with PHA), the transformed T cell lines Hut 78 and YT, peripheral blood NK cells, the NK 3.3 cell line, human peripheral blood monocytes, the myelomonocytic cell lines U937 and THP-1, and the tumour cell lines OVCAR-3, HT-29 and IM-9 were subjected to SDS-PAGE, transferred to Immobilon and probed with antisera to Jak-3. b, Jak-3 expression in human T lymphocytes activated with PHA for 0–48 h.

METHODS. Human peripheral blood T lymphocytes, NK cells and monocytes (>97% pure) were obtained by leukapheresis from donors. T lymphocytes were either untreated or treated with PHA ($10 \mu\text{g ml}^{-1}$) for 0–48 h. The procedures used for lysis, electrophoresis and blotting were described previously¹¹. The anti-Jak-3 antiserum was raised against a



synthetic peptide corresponding to the C-terminal region of the Jak-3 protein (amino acids 1,104–1,124)¹¹.

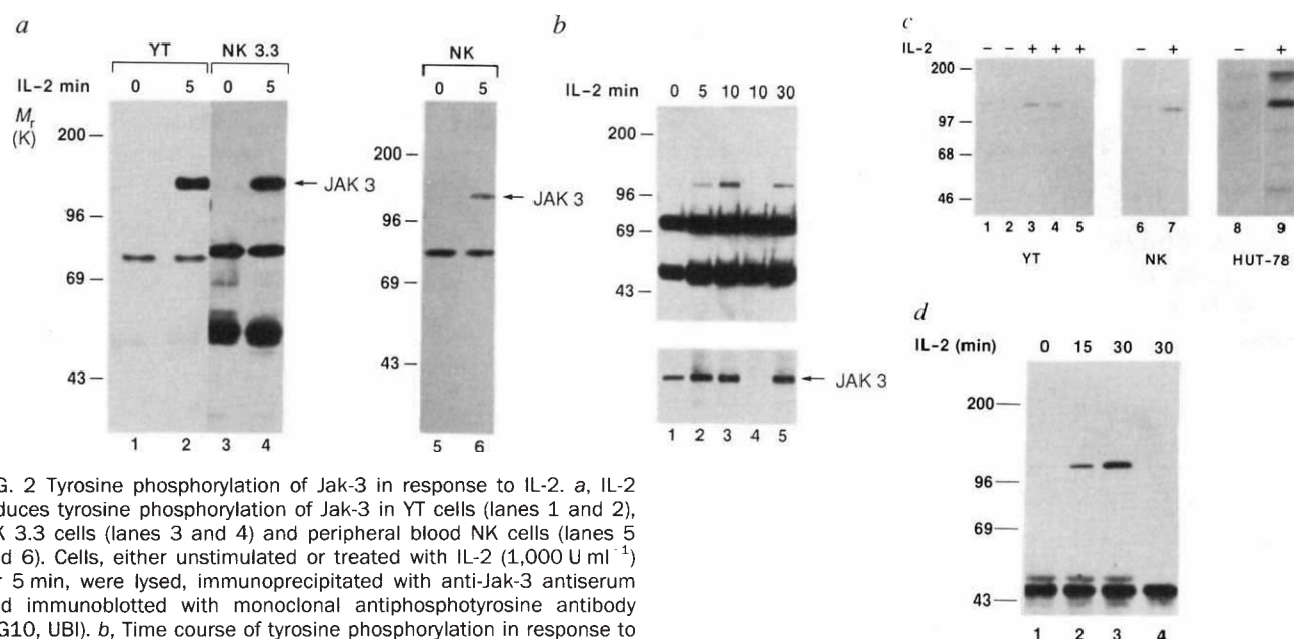


FIG. 2 Tyrosine phosphorylation of Jak-3 in response to IL-2. *a*, IL-2 induces tyrosine phosphorylation of Jak-3 in YT cells (lanes 1 and 2), NK 3.3 cells (lanes 3 and 4) and peripheral blood NK cells (lanes 5 and 6). Cells, either unstimulated or treated with IL-2 ($1,000 \text{ U ml}^{-1}$) for 5 min, were lysed, immunoprecipitated with anti-Jak-3 antiserum and immunoblotted with monoclonal antiphosphotyrosine antibody (4G10, UBI). *b*, Time course of tyrosine phosphorylation in response to IL-2 in Hut 78 cells. Lysates from Hut 78 cells either untreated or treated with IL-2 for 5, 10 or 30 min, were immunoprecipitated with anti-Jak-3 and blotted with antiphosphotyrosine antibody and with anti-Jak-3 (lower panel). Lane 4 shows competition with Jak-3-specific peptide. *c*, IL-2 stimulates Jak-3 *in vitro* kinase activity. YT cells were treated with IL-2 ($1,000 \text{ U ml}^{-1}$) for 0 (lanes 1 and 2), 10 (lane 3) or 20 (lanes 4 and 5) min. *In vitro* kinase activity was measured in anti-Jak-3 immunoprecipitates. Lanes 2 and 5 represent competition with Jak-3-

specific peptide. Jak-3 *in vitro* kinase activity in response to IL-2 stimulation (15 min) was measured in peripheral blood NK cells (lanes 6 and 7) and Hut 78 cells (lanes 8 and 9). *d*, Coimmunoprecipitation of Jak-3 with IL-2R β . YT cells were treated with IL-2 for 0, 15 and 30 min lysed in 0.1% Triton, 0.75% Brij 96 and the lysate immunoprecipitated with anti-IL-2R β chain (lanes 1, 2, 3) or an isotypic matched irrelevant antibody and blotted with anti-Jak-3.

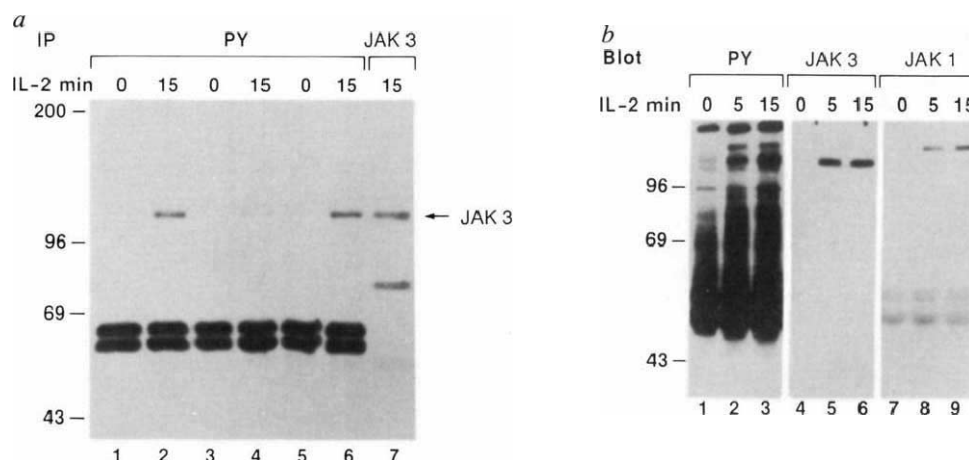
In Fig. 3*b*, immunoblots from a separate experiment were deliberately overexposed to reveal additional substrates. We observed a tyrosyl phosphoprotein induced in response to IL-2 (Fig. 3*b*, lanes 2 and 3) which was larger than Jak-3 (lanes 5 and 6). Immunoblotting confirmed that this protein was Jak-1 (Fig. 3*b*, lanes 8 and 9). Although both Tyk-2 and Jak-2 could be detected in YT cells, no tyrosyl phosphorylation of either protein was observed in response to IL-2. This was determined both by immunoprecipitating with antiphosphotyrosine antibodies and blotting with anti-Jak antibodies and the converse (not shown).

To determine the specificity of Jak-3 phosphorylation, we next examined the response to a number of other cytokines. In YT

cells, Jak-3 was intensely tyrosine phosphorylated in response to IL-2, but only minimal alterations of phosphorylation were seen in response to growth hormone¹⁹ interferon- α (IFN- α) or - γ (Fig. 4*a*), whereas in these cells we could detect tyrosine phosphorylation of Jak-2 in response to IFN- γ ²⁰ (Fig. 4*b*). In addition, Jak-3 was not phosphorylated in response to IL-3, granulocyte macrophage colony-stimulating factor (GM-CSF) or erythropoietin (data not shown) stimuli that have previously been shown to activate Jak-2^{21,22}.

Recently the IL-4 receptor has been shown to comprise the common γ -chain of the IL-2 receptor (γ_c)^{23,24}, suggesting that common signalling pathways may also be used by these cytokines. This prompted us to examine whether Jak-3 was phos-

FIG. 3 IL-2-induced tyrosyl phosphorylation of Jak family kinases. *a*, Jak-3 is the most prominent tyrosine phosphorylated protein in response to IL-2 in YT cells. Lysates from YT cells treated with IL-2 ($1,000 \text{ U ml}^{-1}$) for 0 or 15 min were precleared with either rabbit polyclonal antiserum (lanes 1 and 2), anti-Jak-3 (lanes 3 and 4) or anti-Jak-1 (lanes 5 and 6) before immunoprecipitation and immunoblotting with anti-phosphotyrosine. Lane 7 represents immunoprecipitation with anti-Jak-3 and immunoblotting with anti-phosphotyrosine. *b*, Jak-3 and Jak-1 are phosphorylated in response to IL-2. YT cells treated with IL-2 ($1,000 \text{ U ml}^{-1}$) for 0, 5 or 15 min, immunoprecipitated with anti-phosphotyrosine and immunoblotted with antiphosphotyrosine (lanes 1–3), anti-Jak-3 (lanes 4–6) or anti-Jak-1 (lanes 7–9).



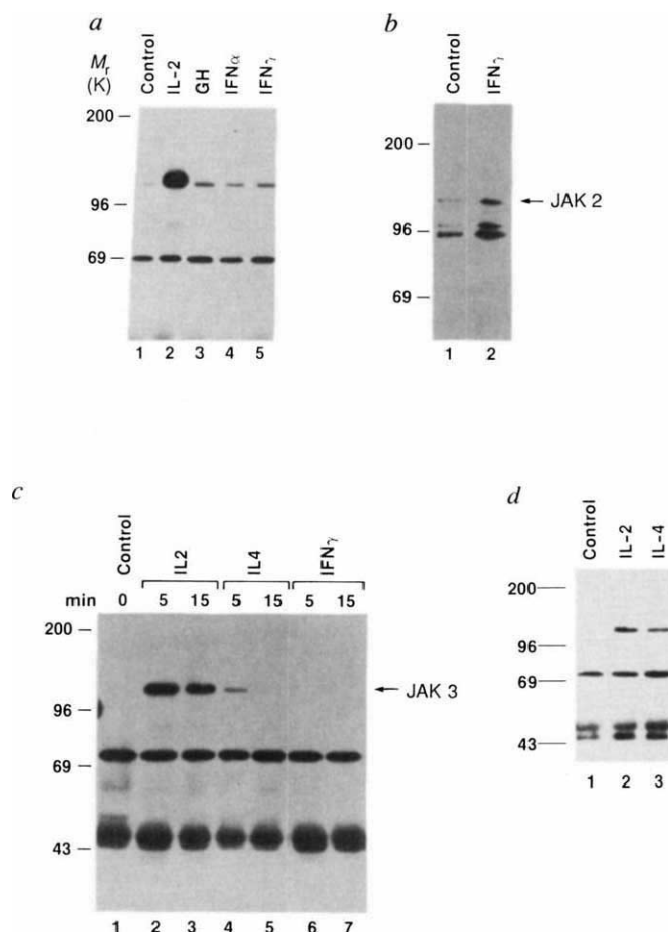


FIG. 4 Phosphorylation of Jak-3 in response to IL-4 but not other cytokines. **a**, YT cells were treated with IL-2 ($1,000 \text{ U ml}^{-1}$), growth hormone (GH; 50 ng ml^{-1}), IFN- α ($1,000 \text{ U ml}^{-1}$) or IFN- γ (500 U ml^{-1}) for 15 min and the lysates immunoprecipitated with anti-Jak-3 and blotted with antiphosphotyrosine. **b**, YT cells were either unstimulated (lane 1) or treated with IFN- γ for 15 min (lane 2), immunoprecipitated with anti-phosphotyrosine and blotted with Jak-2. **c**, NK 3.3 cells rested overnight in 2% serum were challenged with IL-2 ($1,000 \text{ U ml}^{-1}$; lanes 2 and 3), IL-4 (100 U ml^{-1} ; lanes 4 and 5) or IFN- γ (500 U ml^{-1} ; lanes 6 and 7) for 5 or 15 min and the lysates were immunoprecipitated with anti-Jak-3 and blotted with antiphosphotyrosine. **d**, Similarly, PHA-activated (72 h) peripheral blood human T cells were rested overnight in 5% serum before challenge with IL-2 (lane 2) or IL-4 (lane 3) as above.

phorylated in response to IL-4. Consistent with this hypothesis, we found that Jak-3 was tyrosine phosphorylated in response to IL-4 as well as IL-2 in NK 3.3 cells (Fig. 4c), and human peripheral blood T cells (Fig. 4d). Additionally, IL-4 stimulation induced tyrosine phosphorylation of Jak-1 (data not shown).

Two of the IL-2 receptor chains ($\beta + \gamma$) are members of the haematopoietic receptor family²⁵, many other members of which have been shown to couple to Jak family kinases^{26,27}. The data here support the contention that both Jak-3 and Jak-1 probably play important roles in lymphoid activation through the IL-2 and IL-4 receptors. Our findings are in agreement with those of Witthuhn *et al.* (accompanying paper)²⁸. It will be important to determine precisely how these kinases interact with receptor subunits and *src* family protein tyrosine kinases. Additionally, it will be important to determine if Jak-3 and Jak-1 are necessary intermediates in IL-2 signalling and if an IL-2-dependent protein related to signal transduction and activation of transcription exists. Ultimately, IL-2 receptor Jak interactions may become a useful target for pharmacological intervention. □

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Involvement of the Jak-3 Janus kinase in signalling by interleukins 2 and 4 in lymphoid and myeloid cells

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MANY cytokines function through interaction with receptors of the cytokine receptor superfamily. Although lacking catalytic domains, cytokine receptors couple ligand binding to induction of protein tyrosine phosphorylation. Recent studies^{1–10} have shown that one or more of the Janus kinase family members (Jaks) associate with cytokine receptors and are tyrosine phosphorylated and activated following ligand binding. Here we describe a new Jak family kinase, Jak-3, and demonstrate that Jak-3, and to a lesser extent Jak-1, are tyrosine phosphorylated and Jak-3 is activated in the responses to interleukin-2 and interleukin-4 in T cells and myeloid cells. Jak-3 activation requires the serine-rich, membrane-proximal domain of the interleukin-2 receptor β -chain, but does not require the acidic domain that is required for association and activation of *Src* family kinases.

Polymerase chain reaction (PCR) experiments identified a complementary DNA fragment encoding a novel Jak in breast cancer cells¹¹ and, more recently, rat hippocampal neurons¹². Using the initial fragment, cDNAs were obtained from a murine

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1 51
 JAK3 M.....APPSEETPLIPORSCSLSSSEAGALHVLPPRGPQPRLSFSFGDYLAEDLCVRAAKACGILPYHSLFALATEDFSCWFPPSH
 JAK2 MGMACTMTMEATSTSPYHONGDIPGSANSVKQIEPVLQVLYHSLGQAEFYLLKFPSPGEYVAEEICVAASKACGIPYHNMFMALMSETERIWIYPPNH
 JAK1 MQLYLNKEDCNAMAFCAKMRSFKKTEYKQVVPPE..GVEYTFYLLDR...EP..LRLGSGEYTAEECLIRAAQECISPLCHNLFALYDESTKLWYAPNR
 TYK2 MPLRHW.....GMARGSKPVGDGAQPMAMGGLKYLHWAGPGGGEP..WYTFSESSLTAEVCIHIAHKVGIPTPCFNLFLFADAAQVWLPNNH
 Con M-----G--EP--L-F--G-Y-AEE-C--AA--CGI-P--HNLFAL--E---W-PPNH

101 151
 JAK3 LFCIEDVOTQVLYVLRFFYPWF.....GLETCHRFGLRKLDTSAILDHLVLEHLFAQHRSDLVSGRLPV....GLSMKEGGEFLSLA
 JAK2 VFHIDESTRHDILYRIRFFYPHWY.....CSGSSRTYRQVYSGRAEA..PLDDFVMSYLFQWRHDFVHGWIKV....PYTHETQEECLGMA
 JAK1 IITVDKTSRLRHYRMRFYFTNWHGTNDNEQSVWRHSPKKOKNGYEKKRVPPEATPLDASSLEYLFAOGQYDLIKFLAPIRDPKTEQGGHDIENECLGMA
 TYK2 ILEIPRDASLMLYRIRFFYRNWHGMNPREPAYERGPGPGTEASSDOT...AQGMQLLOPASFEYLFEOGKHEFVNDVASLWELSTEEIHHFKNESLGMW
 Con I--I-----L-YR-RFFY--W-----LLD----EYLFQ--D-V-----H---E-LGMA

201 251
 JAK3 VLDLAQMAREQAQRPGELLKTVSYKACLPPSLRDVIOGQNFVTRRRIR...RTVYLALLP...CGRLPGRPYALMAKYILDRLHPAATTETFRV....
 JAK2 VLDMMRIAKEKDQTPLAYVNSYSYKTFLLPKCVAKIQQDYHILTRKIRIRYRFRRIQQFSQ...CKATARN...LKLKYLINLETLSQAFYFEOFEV....
 JAK1 VLAISHYAMKKMQLELPKDISYKRYIPETLNKSLIRQRNLLTRMRINNVKDFLKEFNNTICDSSVSTH..DLKVYKYLATLETTLTKHYGAEIFETS...M
 TYK2 FLHLCHLALRHGIPLEEVAKTSTFKDCIPRSFRRIRIQHSALTRLRLRNVRFRFLRDFQP...GRLSQ...MVMYKYLATLERLAPRFGTERVPPVCHLR
 Con VL-----A-----E--K--SYK--P--R--I-----LTR-RIR--FR-F--F-----C-----L--KYL--LE-L-----TE-F-V-----

301 351
 JAK3GL...PGAQEEPGL...LRVAGDNGIPW.....SS...ND.ELF.....QT.....FCDFP
 JAK2KESARGPSGEEIFAT.....ITGNGGIQW.....SRGKHKESETLTEDVQL.....YCDFP
 JAK1 LLSSENELSRCHSND.....SGNVLYEVMVTGNLGIQWRQKPNVVPVEKEN.....KLKRRKLEYNKKKKDDERNLAREE..WNWFSYFP
 TYK2 LLAQAEGEPCYTRDSGVAPTDPGPESAAGPPTHEVLVTGTGTGGIOWGVVEEVKEEGSSGSGRNPQASLFGKKAKAHKAFDADRPKPRELWAYFCDFP
 Con -----P-----VTG-GGIQW-----S-----FCDFP

401 451
 JAK3 EIVDVSINQAPRVGPAGEHRLVTVMRDMGHILEAEFFPLPEALSFVALVDGYFRILCDSRHYFCKEVAPPRLLEEEADVCHGPIITLDAIHKLLKAAGSLP
 JAK2 DITDVSIKQANQ.ECSNESRIVTVHKQDGKYLEIELSSLEKALSFVSLIDGYYRLTADAHYLCKEVAPPVLENIHNGCHGPIISMOFAISKLKAGNQT
 JAK1 EITHIVIKE.....SVYSINKQDNKNMELKLSSEREALSFVSLVDGYFRILTADAHYLCEDVAPPLIVHNIONGCHGPICTEYAINKLRQEGSEE
 TYK2 DITHVVLKE.....HCVSIHRQDNKCELELSPSRAAALSFFVSLVDGYFRILTADSSHYLCHEVAPPRLVMSIRDGIHGPLLEFPVQAKLR...PED
 Con -I--V-IK-----V----QD-K-LE--L-S--EALSFVSLVDGYFRILTAD--HYLC-EVAPP-----I---CHGPI---FAI-KL--G---

501 551
 JAK3 GTYILRRSPQDYDSFLLTA.CVQTPLGPDYKGLIRQD...PSGAFSLVGLSQPHRSLRELLAACWN..SGLRVDGAALYLTSCCAPRPKEKSNLIVVR
 JAK2 GLYVLRCSPKDFNKYFLTF.AVERENVIEYKHCLITKN.....ENGEYNLSGTRKRNFSNLKDLLN.CYOMETVRSDSITTFQTKCCPPKPKDKSNLLVFR
 JAK1 GMYVLRWCTDFDNLMTVTCEKSE.VLGGOK.QFKNFQIEVQKGRYSLHGSMDHFPSLRDLNMH..LKKQILATDNIISFVLKRCCKCPKPREISNLLVA
 TYK2 GLYLHWSTSHPYRLILVA..QRQAPDGMQSLRLRKFPTEQQDAFVLEGWGRSPSVRELGA..LQGLLRAGDDCFSLRRCCPLQPGGETSNLIM.
 Con G-Y-LR-S--D-----LT-----L-----G--L-G--F-SLR-L-----LR-D--F-L--CC-P-P-E-SNL-V--

601 651
 JAK3 .RGCNPAAPAGCSPSCCALTQLSFHTIPTDSLEWHENLHGHSFTKIFRGSRR.....VVD.GETHOSEVLLKVMDSRHRCNMESE
 JAK2 TNGISDVQISPTLQRHNNVNQMVFKIRNEDLIFNESLGGGTFTKIFKGVRR.....VGDYGGQLHKEVLLKVLDAKARNYSSES
 JAK1TKKAQEWQPVYMSQLSFDRILKDDIOGHELGRGTRTHIYSGTLLDYKDEEGIAEEKI.....KVILKVLDPDSHRDILSLAF
 TYK2RGARASPTLNLQSLSFHRVDQKEITQLSHLGGGTRTNVYEGRLV..EGSGDPEEGKMDDEDLPVGRDRQDELRVVLKVLDPDSHMDIALAF
 Con -----QLSFH-I-----E-LG-GT-T-I--G--R-----V-----V-LKVLDP--HR-----F

701 751
 JAK3 LEAASLMSQVSPHYPLVLLHGVCMDG.SIMVQEFVYLGIDMYLRKRGHVLSASWKLQVTKQLAYALNYLEDKGLPHGNVSGAPKVLAREGG..DGNPPF
 JAK2 FEASAMMSQLSHKHLVNLGYGVCVCGEENILVQEFVFKGSLDTYLLKNKNSINILWLKLGVAQLAWAMHFLKESLIHGNVCAKNILLIREEDRRGTNPPF
 JAK1 FEASAMMRQVSHKHLVYLVGYGCVRDVENIMVEEFVEGGPLDLFMHRKSDALTTPWKFVKVAKQLASALSYLEDKLVHGNVCTKNLLAREGID..SDIGPF
 TYK2 YETASLMSQVSHTHLAFVHGVCRGPNESMTLYEYHGVPLDWLRERHGVPMAMVVAQQLASALSYLENKLVHGNVCGRNLILARLGLA..EGTSPF
 Con -EAAS-MSQVSH-HLV--GVCV-G-ENIMV-EFV--G-LD-----WK--VA-QLA-AL-YLE-K-L-HGNVC--N-LLAREG---G--PF

801 851
 JAK3 IKLSDPGVSPTVLSLEMLTDRIPIWYAEPL.QEAQTLCLAEADKWGFATTWYEFQRPAAHITSLEPAKKLKFYEQQQLPAKWTELAGLITQCMAYDPG
 JAK2 IKLSDPGISITVLPKDILOERIPWYVPECI..ENPKNLNLTADKWSFGTTLWEICSGGDKPLSALDSQRKLQFYEDKHQLPAPKWTELANLNNCMYDPE
 JAK1 IKLSDPGIPYVSLTRQECIERIPWIAPECV..EDSKNLSVAADKWSFGTTLWEICSGGDKPLSALDSQRKLQFYEDKHQLPAPKWTELANLNNCMYDPE
 TYK1 IKLSDPGIGLALSREERVERIPWLAPECLPGGANSLSAMDKWGFATLLEICFDGEAPLQSRSPSEKEHFYQROHRLPEPSCPLATLTSQCLTYEPT
 Con IKLSDPGI--VL-----ERIPW-APEC-----L--A-DKW-FG-TLWEIC--G--PL-----K--FYE-----LP-P--ELA-L---CM-Y-P-

901 951
 JAK3 RRPFSFRAILRDLNLITSDYELLSDPTPGIPSPRDELCAVAGQLYACQDPAIFEEERHLKYISLLGKGNFGSVYELCRYDPLGDNTPGLVAVKQLQ..HSVPD
 JAK2 RPAFRAVIRDLNLFPTDYELLTENDM..LPNMRIGALGF..SGAFEDRPTQFEERHLKGLQQLGKGNFGSVEMCRYPLODNTGEVAVKQLQ..HSTEE
 JAK1 QRPFFRAIMRDINKLEEON..PDIVSEKOP.....TTEVDPTHEKFRFLKRIIDLGEHGFVKVELCRYDPEGDNTEQGVAVKSLKPESGGN
 TYK2 QRPFSFTILRDLTRVOPHNLDVLTNRDSP.....A..VGPTTFHKRYLKKIRDLGEHGFVKVSLCYDPTNDGTGEMVAVKALKADCGPQ
 Con -RP-FRAI-RDLN-L-----P-----PT-FE-R-LK-I--L-G-G-FG-VELCRYDP--DNTGE-VAVK-L---S---

1001 1051
 JAK3 QQRDFQREIQILKALHSDFIKYRGVSYGPGRQSLRLVMEYLPSCGLRDLQRHRG..LHTRDLLFAWQICKGMEYLGARRCVHRDLAARNILVESEAHV
 JAK2 HLRFDERIEIILKSLQHDNIYKYKGVCYSAGRRLRLIMEYLPYGSRLDYLOKHKEIRIDHKKLLOYTSQICKGMEYLGTRKYIHRDLATRNILVENENRY
 JAK1 HIADLKKIEIILRNLHYENIKYKICMEDGGNGIKLIMEFLPSGSLKYLEPLKNNKINLKOQLKYAIOICKGMDYLGSRQVYHRDLAARNILVESEAHV
 TYK2 HRSQWKEQIDILRLTYHEHIKYKGCCEQGEKSLQVMEYPLGSLRDYLRHS...IGLAQLLLFAQQICEGMAYLHAHDYIHRDLAARNVLLDNDRLV
 Con H--D---E-I-L--L-H--IVKYKG-C--G--L-L-MEYLP-GSLRDYL--H---I---L--A-QICKGM-YLG--Y-HRDLAARN-LVE-E--V

1101 1151
 JAK3 KIADFGLAKLLPLGKDYVYVREPQSPFIWYAPESLSDNISFRQSDVWSFGVVLVELFTYCDKSCSPSAEFLRMGMFEREGPPLC..RLELLAEGRRLPP
 JAK2 KIGDFGLTKVLPODKKEYYKKEPESPIFWYAPESLSDNISFRQSDVWSFGVVLVELFTYCDKSCSPSAEFLRMGMFEREGPPLC..RLELLAEGRRLPP
 JAK1 KIGDFGLTKAETDKKEYYVTKDDRSPVFWYAPESLSDNISFRQSDVWSFGVVLVELFTYCDKSCSPSAEFLRMGMFEREGPPLC..RLELLAEGRRLPP
 TYK2 KIGDFGLAKAVPEGEHYRVEDGSPVFWYAPESLSDNISFRQSDVWSFGVVLVELFTYCDKSCSPSAEFLRMGMFEREGPPLC..RLELLAEGRRLPP
 Con KIGDFGL-K--P--KEYY-V-E-G-SP-FWYAPE-L--KF--ASDVWSFGV-VLYEL-TYCD-S-SP--FL-MISG--GOM-V-SKL-R-G-M-LP-

1201
 JAK3 PPTCPTVEQLMQLCWAPEPHDRPAFATLSPOLDPLW.RG.....RPG*
 JAK2 PEGCPDEIYVIMTECWNNNVSQRPFRDLSEF.....WIKS.....GTV*
 JAK1 PPNCPDEYQLMRKCEWFQPSNRITTFONLIEGFALLK*
 TYK2 PDKCPCEVYHLMKNCWEATEASFRPTFENLIPILKTVHEKYQGQAPSVFVSYC*
 Con P--CP-EVY-LM--CW-----S-RP-F--L-----

◀ FIG. 1 Amino-acid sequence (single-letter code) comparisons of the Jak family of kinases. The amino-acid sequences of murine Jak-1 (O.S. and J.N.I., unpublished data), murine Jak-2 (ref. 4) and human Tyk-2 (ref. 13) are compared with the murine Jak-3 sequence. Alignments were initially made by computer analysis and were subsequently aligned by inspection. Gaps were introduced to optimize alignment. The consensus alignment indicates positions in which 3 or 4 of the sequences have an identical amino acid. The sequence of murine Jak-3 has been deposited in GenBank, accession number L329555. METHODS. PCR amplification with degenerate kinase domain primers and cDNA from primary breast cancer tissue was used to identify novel kinases as previously described¹¹. The PCR fragment (TK5) was used to screen a mouse pre-B-cell cDNA library²⁷ by standard techniques. Four cDNA clones were obtained, one of which was near the size of the transcript detected by northern blots. The nucleotide sequence was determined by dideoxynucleotide, chain termination sequencing²⁸ in both directions.

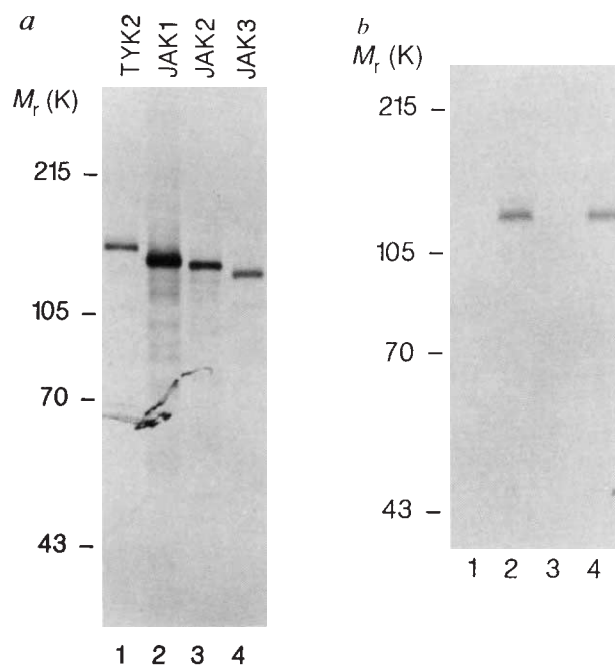


FIG. 2 *In vitro* translation of cDNAs for Jak family members and characterization of antisera. *a*, cDNAs for murine Jak-1, Jak-2 and Jak-3 and human Tyk-2 were transcribed and translated *in vitro* using the Promega (Madison, WI) TNT T3 coupled reticulocyte system and labelled with [³⁵S]methionine as described previously⁴. The reaction products were resolved by SDS-PAGE and the proteins detected by autoradiography. *b*, Characterization of antisera against Jaks. The ³⁵S-labelled Jak-3 protein was used in immunoprecipitation reactions with a preimmune serum (lane 1), an anti-peptide antiserum against Jak-3 (lane 2), the antiserum against Jak-3 in the presence of excess peptide (100 µg ml⁻¹) to which the antiserum was raised (lane 3) or an irrelevant peptide (lane 4). The anti-peptide antiserum was raised against the peptide AKLLPLDKDYVVRPG by previously described techniques⁴. The crossreactive, anti-peptide antiserum was made against a synthetic peptide derived from Tyk-2 (SPSEKEHFYQAQHRLEPESC).

B-cell library. The longest (3.8 kilobases (kb)) contained an open reading frame encoding a Jak-related protein of relative molecular mass 122,600 (M_r 122.6K) with 1,099 amino acids (Fig. 1), which we termed Jak-3. Murine Jak-3 has 47%, 36% and 36% amino-acid identity to murine Jak-2 and Jak-1 and human Tyk-2, respectively. Jak-3 contains a tyrosine kinase catalytic domain and a more amino-terminal kinase-like domain. In addition, there is similarity between the N-terminal blocks of Jak-3 and the other Jaks.

Translation of Jak-3 cDNA *in vitro* (Fig. 2) gave a 120K product which could be distinguished from other Jaks by size, consistent with the predicted sizes. The *in vitro* translated proteins were used to determine antiserum specificity (Fig. 2*b*). Antiserum against a Jak-3 kinase domain peptide immunoprecipitated Jak-3 (Fig. 2*b*, lane 2) but not Jak-1, Jak-2 or Tyk-2 (data not shown). Precipitation was not seen with preimmune serum (Fig. 2*b*, lane 1) and was competed by the immunizing peptide (lane 3) but not an irrelevant peptide (lane 4). Similarly, antisera against Jak-1 or Jak-2 (ref. 4) did not immunoprecipitate Jak-3 (data not shown). Last, an anti-peptide antiserum against the region between the kinase domains of Tyk-2 was crossreactive and recognized Jak-3 and Jak-1 as well as Tyk-2 but only weakly recognized Jak-2. A commercial Tyk-2 antiserum (Santa Cruz Biotechnology Inc.) did not crossreact with Jak-1, Jak-2 or Jak-3.

In contrast to the ubiquitous expression of the previous Jaks^{4,13,15}, Jak-3 expression is restricted (Fig. 3). A 4-kb transcript was highly expressed in an interleukin-2 (IL-2)-dependent cytotoxic T cell (CTLL) and myeloid cells but not fibroblasts. Among tissues, transcripts were highest in spleen and less in liver, kidney, lungs and heart but not in brain or testes (data

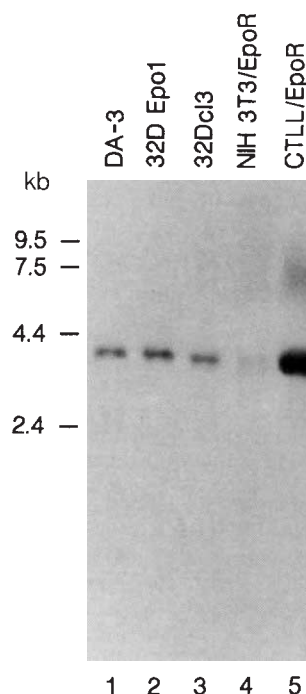


FIG. 3 Jak-3 expression in murine cell lines. RNA was prepared from the indicated cells by previously described techniques⁴ using about 15 µg of total RNA. The RNAs included (1) an IL-3-dependent myeloid cell line (DA-3); (2) an IL-3-dependent myeloid cell line, 32D Epo1 (ref. 29); (3) an IL-3-dependent myeloid cell line, 32Dcl3 (ref. 29); (4) NIH 3T3 fibroblasts transfected with the wild-type erythropoietin receptor (EpoR); and (5) a clone of an IL-2-dependent cytotoxic T-cell line stably transfected with the Epo receptor, CTLL/EpoR. The position of migration of RNA standards are shown. The single Jak-3 transcript migrates with an apparent size of 4.0 kb. The probe consisted of a 1-kb SstI fragment of the cDNA, labelled by random priming.

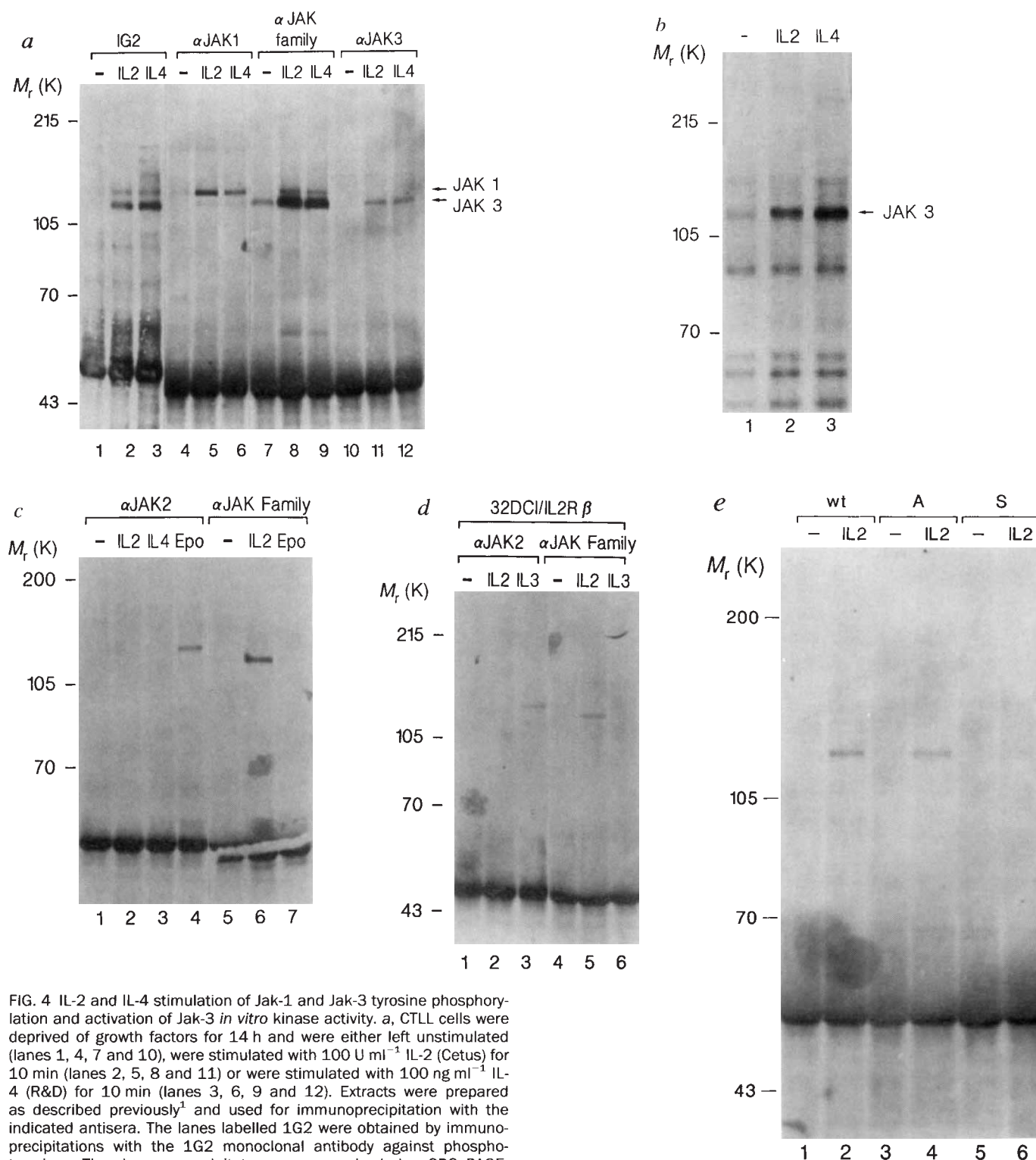


FIG. 4 IL-2 and IL-4 stimulation of Jak-1 and Jak-3 tyrosine phosphorylation and activation of Jak-3 *in vitro* kinase activity. **a**, CTLL cells were deprived of growth factors for 14 h and were either left unstimulated (lanes 1, 4, 7 and 10), were stimulated with 100 U ml⁻¹ IL-2 (Cetus) for 10 min (lanes 2, 5, 8 and 11) or were stimulated with 100 ng ml⁻¹ IL-4 (R&D) for 10 min (lanes 3, 6, 9 and 12). Extracts were prepared as described previously¹ and used for immunoprecipitation with the indicated antisera. The lanes labelled 1G2 were obtained by immunoprecipitations with the 1G2 monoclonal antibody against phosphotyrosine. The immunoprecipitates were resolved by SDS-PAGE, electrophoretically transferred to nitrocellulose and the membranes were probed with the 4G10 monoclonal antibody (UBI) against phosphotyrosine. **b**, CTLL cells were deprived of growth factors for 14 h and were either unstimulated (lane 1), stimulated with IL-2 (lane 2) or stimulated with IL-4 (lane 3), as above. Extracts were prepared and used for immunoprecipitation with the Jak-3/Jak-1 crossreactive antipeptide antiserum against Tyk-2. The immunoprecipitates were used in *in vitro* kinase assays as described previously¹ and the products resolved by SDS-PAGE and visualized by autoradiography. **c**, CTLL cells transfected with the Epo receptor were deprived of IL-2 for 14 h and were either left unstimulated (lanes 1 and 5), were stimulated with 100 U ml⁻¹ IL-2 (lanes 2 and 6), 100 ng ml⁻¹ IL-4 or 10 U ml⁻¹ of Epo (lanes 4 and 7). Extracts were prepared and blots obtained as above and probed with the 4G10 monoclonal antibody against phosphotyrosine. The posi-

tions of migration of standards are shown on the left. **d**, 32Dc13 cells transfected with the human IL-2- β -receptor chain (32D/IL2R β) were deprived of IL-2 for 14 h and either not stimulated (lanes 1 and 4) or stimulated with 100 U ml⁻¹ IL-2 (lanes 2 and 5) or 10 U ml⁻¹ IL-3 (lanes 3 and 6). Extracts were made, resolved by SDS-PAGE and transferred to filters as above. The filters were probed with the 4G10 monoclonal antibody to phosphotyrosine. **e**, 32Dc13 cells transfected with the wild-type human IL-2- β -receptor chain, or the A or S mutant receptors treated as in **d**.

METHODS. Cells were collected and extracts prepared in 1.0% Triton X-100 as described previously¹. Cell extracts from 2×10^7 cells were used for immunoprecipitations with the designated antisera. The conditions for the *in vitro* kinase assays were as described¹.

not shown). Consistent with previous results¹¹, Jak-3 is expressed in breast-tissue-derived cell lines (K.S.L. and E.T.L., unpublished observations).

To assess Jak-3 in signalling, cytokine-induced tyrosine phosphorylation was examined. Phosphorylation was not seen with erythropoietin, IL-3, granulocyte macrophage colony-stimulating factor (GM-CSF), G-CSF, interferon- α (IFN- α), IFN- γ or IL-6 (data not shown), but was seen in IL-2- or IL-4-stimulated cells. In CTLL cells, IL-2 and IL-4 induced tyrosine phosphorylation of several proteins (Fig. 4), including proteins of 120K and 130K, as recently shown¹⁶. IL-2 and IL-4 induced tyrosine phosphorylation of Jak-3 (α Jak-3), which comigrated with the major 120K substrate, and Jak-1 (α Jak-1), which comigrated with the 130K substrate. No Jak-2 or Tyk-2 phosphorylation was detected (data not shown). Last, the Jak-3/Jak-1-cross-reactive Tyk-2 antiserum immunoprecipitated proteins that comigrated with Jak-1 and Jak-3 but not Tyk-2. Phosphorylation was detectable within 1 min, peaked at 20–30 min and subsequently declined (data not shown).

Cytokine-induced tyrosine phosphorylation of Jaks activates their *in vitro* kinase activity^{1,2,4,7,9}. Therefore, we examined the effects of IL-2 or IL-4 on kinase activity. No kinase activity was detected in immunoprecipitates of Jak-1 or immunoprecipitates of Jak-3 obtained with the Jak-3-specific antiserum (data not shown). However, the Jak-3 antiserum is against a peptide containing the autophosphorylation site (KDYV; single-letter amino-acid code) and could interfere with activity. Therefore immunoprecipitates obtained with the Jak-1/Jak-3 crossreactive antiserum were examined. With these, IL-2- and IL-4-induced *in vitro* kinase activity was readily detected (Fig. 4b) and the single phosphorylated protein comigrated with Jak-3. No detectable Jak-1 phosphorylation was seen. Amino-acid analysis of phosphorylated Jak-3 detected exclusively phosphotyrosine (data not shown).

To assess the specificity of Jak-3 activation, we examined CTLL cells expressing the erythropoietin receptor, which shares homology in the cytoplasmic domain with the IL-2 receptor β -chain¹⁷. Transfected CTLL cells express levels of high-affinity erythropoietin receptors comparable to transfected myeloid cells (O.M. and J.N.I., unpublished data). Erythropoietin, but neither IL-2 nor IL-4, induced tyrosine phosphorylation of Jak-2 (Fig. 4c). Conversely, IL-2, but not erythropoietin, induced phosphorylation of Jak-3.

The IL-2 receptor consists of α -, β - and γ -chains¹⁸. The γ -chain is also used in the IL-4 receptor^{19,20} and is essential for function. The β -chain is also essential and contains two functional domains. An acidic domain is required for association and activation of p56^{lck}, p59^{fyn} or p53/56^{lyn}¹⁸ but is dispensable for mitogenesis. In contrast, the membrane-proximal, serine-rich domain is required for mitogenesis. To assess their role in Jak-3 activation, the wild-type and receptors lacking the acidic (A-mutant) or serine-rich (S-mutant) domains were introduced into IL-3-dependent 32Dcl3 (Fig. 4d, e). Cells expressing the wild-type receptor, or the A-mutant, responded to IL-2 but not cells expressing the S-mutant. IL-3 stimulation of cells expressing the wild-type receptor resulted in only Jak-2 phosphorylation. In contrast, IL-2 stimulation induced Jak-3 phosphorylation, but not Jak-2 or Tyk-2, and activated Jak-3 *in vitro* kinase activity (data not shown). Of note, Jak-1 was not detectably phosphorylated in any of these experiments. Last, IL-2 stimulation of cells expressing the A-mutant-, but not the S-mutant-, induced activation of Jak-3.

The results demonstrate that IL-2 and IL-4 consistently activate Jak-3 and, in CTLL cells, also activate Jak-1. Activation of two Jaks is required for IFN signalling^{7,8}; thus, it will be important to determine whether activation of both Jak-1 and Jak-3 is required for a T-cell response. Activation of Jak-3 requires the membrane-proximal, serine-rich region of the IL-2 receptor β -chain and thus correlates with mitogenesis. However, additional approaches are needed to determine if Jak-3 is essential for IL-

2 signalling. In several cytokine receptors, the membrane-proximal, box1/box2 motifs bind Jaks. The presence of this motif in the IL-2 β -chain suggests that Jak-3 associates with the β -chain. Consistent with this, Jak-3 co-immunoprecipitates with the IL-2R complex (Johnston *et al.*, accompanying manuscript)³⁰ and previous studies¹⁶ identified an IL-2-induced tyrosine phosphorylated 116K protein crosslinked to the β -chain which might be Jak-3. In addition to Jaks, cytokines activate transcription factors of the signal transducers and activators of transcription (STAT) family^{21–25} and related proteins are induced by IL-4 and IL-2²⁶ (W. Thierfelder and J.N.I., unpublished data). Thus, the identification of Jak-3 in IL-2 and IL-4 signalling supports the general concept that cytokine receptor superfamily members couple ligand binding to activation of Jaks which, in turn, activate STATs as a primary signalling pathway. □

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CORRECTION

Binding of phosphatidylinositol-3-OH kinase to CD28 is required for T-cell signalling

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Nature **369**, 327–329 (1994)

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Complex machinations

New strategies for automated genotyping and linkage analysis will bring greater power to the study of complex hereditary disorders.

SLOWLY but surely, the genes underlying many of the most common and serious genetic diseases are succumbing to modern cloning techniques and (on occasion) good guesswork. Some of these achievements have been the culmination of epic struggles lasting years, such as the discoveries of the genes for Huntington's disease and polycystic kidney disease (PKD). Others have been remarkable for the astonishing pace with which the initial discovery of linkage has been followed by the isolation of the gene itself, notably the two loci identified for hereditary non-polyposis colon cancer (HNPCC). Like a typical summer at the movies, we can look forward to a season dominated by gene sequels. Alzheimer's disease II, PKD II, HNPCC III and so on are to come, while we await, of course, the *Jurassic Park* of releases — identification of the gene for hereditary breast and ovarian cancer, *BRCA1*.

As the number of high-profile, single-gene defects yet to be found starts to dwindle, attention is turning to the far more daunting matter of understanding complex — and common — polygenic diseases, in which several genetic loci contribute to the disorder. Examples include diabetes, hypertension, cancers and a wide range of mental illnesses. There has been encouraging progress in defining mouse (and rat) models for some of these disorders, but extrapolating such advances to humans has proved difficult. In many cases, genes incriminated in animal models may simply not be involved in the corresponding human disease.

Although humans lack the numerous advantages of their murine cousins for rapid, informative genetic analysis, steps can be taken to accelerate the search for human genes that control, or at least influence, the development of complex disorders. One is simply to increase the number of highly polymorphic, microsatellite markers available for linkage analysis. J. Weissenbach and

colleagues at Génethon recently described more than 2,000 such markers¹, well on their way to their final goal of 5,000 or so. Another tack is to speed up the ways in which a subset of existing markers can be typed in family samples and translated into linkage analysis. In the latest issue of *Nature Genetics*², J. Todd and colleagues at the University of Oxford describe such a resource.

What Todd's team has done is to adapt some 250 microsatellite markers for use in a commercial, fluorescence-based automated DNA sequencer to simplify the tedious task of typing the allele sizes of hundreds of markers for dozens of family samples. These markers, chosen for their even distribution across the genome and high informativeness, are divided into 39 chromosome-specific sets of 7–10 markers apiece. Each marker within a set recognizes a different range of band sizes, meaning that several markers can be run on a single lane of the sequencer. Two computer programs then size the bands and tabulate the information, giving the peak allele size for each marker in each individual. In this way, thousands of genotypes can be calculated per day per sequencing machine — Todd's group says that it has generated more than 100,000 genotypes in the past six months alone.

There are still improvements that can be made to the software and the design and location of the markers. Nevertheless, the ability to screen the entire human genome rapidly and efficiently should have a dramatic impact on human genetics generally. Todd's group is currently engaged in the search for human loci involved in the aetiology of type 1 diabetes, having shown that some ten genes are involved in the murine model of the disease³.

For all that there has been gratifying progress in mapping the human chromosomes and identifying more and more disease genes, we still have only the vaguest notion as to how many genes there are in the genome. Most published estimates willingly tolerate a margin of a factor of two, citing 50,000–100,000 as the most likely number. Indeed, some have put the figure as anywhere between 30,000 and 300,000 genes. Now, however, a variety of experimental approaches is being brought to bear on the question. C. Fields *et al.* at The Institute for Genomic Research believe that a more accurate tally is possible, and

describe their approach in a News and Views article⁴.

Calculations of the total number of CpG islands (which are often closely associated with genes) in the human genome, and extrapolation from the observed gene density encountered when large (~100 kilobase) segments of human genomic DNA have been sequenced, point to surprisingly similar numbers of total genes. These estimates are supported by Fields and colleagues' own analysis of thousands of partial sequences of complementary DNA (or expressed sequence tags), which they have matched against the known genomic sequences present in the public databases to gauge the probable total number of human transcripts. Although each estimate relies on some key assumptions that are open to debate, Fields *et al.* suggest that all three lines of inquiry point to the true number of human genes lying between 60,000 and 70,000, or about one gene every 40–50 kilobases.

A third paper in July's *Nature Genetics*⁵ shows that patience is indeed a virtue: T. Jacks and coworkers at the Massachusetts Institute of Technology describe an interesting phenotype, in mice lacking the equivalent of the human neurofibromatosis type 1 (*Nf1*) gene, that took some time to emerge. Homozygous mice completely devoid of *Nf1* suffer from very severe developmental abnormalities⁶. But to gain insights into human neurofibromatosis (a dominant disorder characterized by benign neurofibromas), Jacks's team had to observe their heterozygous *Nf1*-deficient mice for 18 months before the phenotype manifested itself. The mice did not acquire the trademark neurofibromas. But they were predisposed to a range of tumours found in NF1 patients, including pheochromocytomas, and half of those examined were associated with loss of the wild-type *Nf1* allele. Not for the first time, a mouse model produced by targeted gene disruption has not quite lived up to expectations; nonetheless, these heterozygous mice should prove very valuable in the study of NF1.

Kevin Davies

Kevin Davies is the editor of Nature Genetics.

Also in this month's *Nature Genetics*: improved vector design for cystic fibrosis gene therapy; CpG island distribution in mammalian chromosomes; the murine T (*Brachyury*) gene family; mapping of recessive genes for polycystic kidney disease and amyotrophic lateral sclerosis; and germline mutations in the thyrotropin G-protein receptor gene in hyperthyroidism.

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Looking at labels

New in the marketplace this week — recombinant firefly luciferase, a new fluorescent reagent for fatty acid analysis, fluorescently labelled tubulin and an assortment of hardware options.

ASSOCIATES of Cape Cod, a licensed manufacturer of *Limulus* amebocyte lysate (LAL), has added pyrochrome to its product line for the **detection of bacterial endotoxin** (Reader Service No. 100). Pyrochrome is a single-step chromogenic LAL which can be used in three types of assays — kinetic, endpoint or diazo-coupled. The latter is said to allow more accurate testing of samples such as blood, blood products (serum, plasma) and other samples which may interfere due to the yellow colour.

New from Promega — **recombinant firefly luciferase** (Reader Service No. 101). The enzyme has a stated specific activity of 3.3×10^{10} relative light units per milligram of enzyme and a purity of greater than 95 per cent by gel electrophoresis. The company also offers a range of other bioluminescence reagents, including D-luciferin, the pGEM-luc cloned firefly luciferase cassette, luciferase reporter vectors and luciferase assay systems for bioluminescence reporter gene studies.

Stratagene's new FLASH CAT non-radioactive **chloramphenicol acetyltransferase assay kit** (CAT assay kit) is designed to provide a rapid assay for the *in vivo* study of *cis* and *trans* transcriptional elements in mammalian systems and lower eukaryotic and prokaryotic cells (Reader Service No. 102). The FLASH CAT kit's protocol is similar to that of the ^{14}C -CAM (chloramphenicol)-based assays, but does not require radioactive materials, film, fluors or a scintillation counter. The protocol is said to take 3.5 h and has a stated sensitivity of 3–10 pg. Results can be analysed using standard instant photography, a still video imaging system such as Stratagene's Eagle Eye II sys-

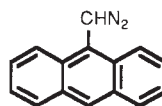
tem, or a fluorometer. The kit is available with the CAM substrate or the 1-deoxyCAM substrate for improved linearity. Sufficient reagents for 100 reactions are included.

■ Fluorescent reagents

ADAM (9-anthryldiazomethane) is a new **fluorescent and ultraviolet reagent for fatty acid analysis** from Kamiya Biomedical (Reader Service No. 103). It reacts with carboxylic acids at room temperature to give fluorescent esters that can be quantified with HPLC; heating or a catalyst are not required. Most organic solvents can be used as a reaction medium. The reagent can be used to detect picomole quantities of fatty acids; it can also be used as a way routinely to measure microgram amounts of oxalic acid in biological samples such as urine. In addition, the company offers a new line of **fluorescent probes and dyes** for use in analytical biochemistry, clinical analysis, biomedical research and biotechnology. Included among these are DNA/RNA stains, fluorescent chelators and ion probes, fluorescent labels, fluorescent lipophilic and membrane probes, and fluorescent protein kinase C probes.

ADAM

(9-Anthryldiazomethane)



Fluorescent reagent for fatty acid analysis.

For researchers studying microtubule dynamics *in vitro* and *in vivo*, Molecular Probes now offers tubulin conjugated with the fluorescent dye tetramethylrhodamine. The **fluorescently-labelled tubulin** is prepared using a method that yields approximately four tetramethylrhodamine molecules per tubulin heterodimer (Reader Service No. 104). Molecular Probes says that its fluorescent tubulin has been subjected to three cycles of temperature-dependent polymerization/depolymerization after labelling to select functional subunits. Conjugated tubulin can be used directly to observe cell cycle-dependent microtubule dynamics and mitotic spindle morphogenesis. Unlabelled tubulin and labelled colchicines are also available to aid researchers in probing the cytoskeleton.

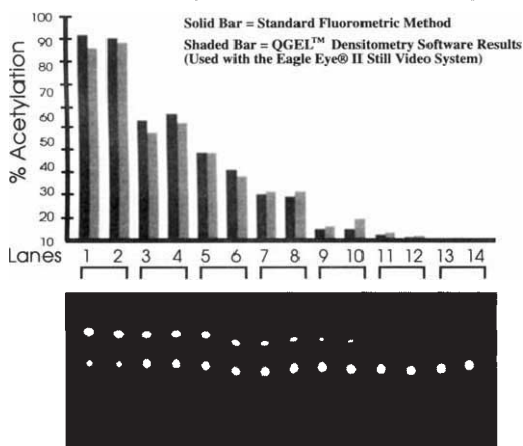
Mikralgen has introduced **natural fluorescent molecules** for use as fluorescent markers in fluorescence immunoassays, fluorescence

microscopy, multiple labelling, immunohistochemistry and fluorescence-activated cell sorting (Reader Service No. 105). The line-up includes two high-purity phyco-biliproteins isolated from the axenic culture of two microalgae: C-phycoerythrin from *Spirulina platensis* and B-phycoerythrin from *Porphyridium cruentum*. Research or bulk quantities are available.

Sigma Immunochemicals has a new **kit for the conjugation of fluorescein isothiocyanate (FITC)** to immunoglobulins for immunohistochemistry and immunofluorescence studies using flow cytometry (Reader Service No. 106). Designed for labelling monoclonal and polyclonal antibodies, the FluoroTag kit may also be used to label other proteins, peptide hormones, cytokines and growth factors. Results can be obtained in 2 h. Each kit contains the reagents necessary for at least five conjugations at pH 9, conjugation procedures for optimal labelling of protein in small or large scale, and a column purification set and a protocol.

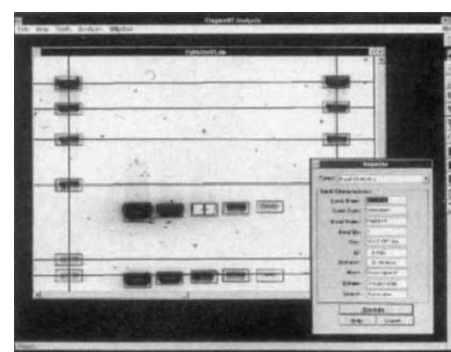
■ Molecular analysis

For researchers wanting to convert from radioisotopic methods to fluorescence, Molecular Dynamics has introduced the FluorKit **DNA fragment analysis system** for fluorescence imaging using the company's FluorImager 575 scanning system (Reader Service No. 107). Stated uses include restric-



Enzyme titration using FLASH CAT assay deoxy-CAM substrate quantified by fluorometry versus QGEL densitometry.

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FluorKit DNA fragment analysis using fluorescence imaging.

tion digests, quantitative PCR, DNA mapping and DNA purification assays. The kit includes standards, stain and an experimental protocol. The high-sensitivity stain, developed by Promega, is said to provide detection limits in the attomole range (similar to radioisotopic methods), and enables staining to be performed *in situ* during electrophoresis. In addition, the company says that fluorescence background of unincorporated stain is low enough that 'wet' gels

can be imaged straight after electrophoresis, without a destaining step.

To avoid the need for repeated freeze-thaw cycles, ICN Biomedical now offers the IsoBlue line of **radiolabelled nucleotides** which can be stored at 4 °C (*Reader Service No. 108*). In addition, the inclusion of an inert dye in the formulation allows visualization of the small aliquots of material routinely used by researchers. The IsoBlue nucleotides have been formulated as a direct substitute for more traditionally formulated products requiring storage at -20 °C.

Clontech offers Biotin-ON phosphoramidite for use in the **production of biotinylated oligonucleotides** directly on an automated DNA synthesizer (*Reader Service No. 109*). Biotin-ON can be used to biotinylate oligonucleotides at multiple sites and, says Clontech, at virtually any position including the 5' terminus. In addition, it is said to conserve the natural distance and structure between internucleotide phosphate groups, thus maintaining the annealing and hybridization efficiency of the oligonucleotide. Biotin-ON contains a dimethoxytrityl group for determination of coupling efficiency, and is soluble in acetonitrile and stable to deprotection with ammonium hydroxide. Also available is a fluorescein-ON phosphoramidite for use in automated oligonucleotide synthesis.

A new **method for labelling DNA**, called the Universal Linkage System, is now available from Kretech Diagnostics (*Reader Service No. 110*). This method uses a platinum derivative as an intermediary ('linker') between nucleic acids and nonisotopic markers, and does not involve any enzymatic steps. Stated applications include the labelling of amplified DNA and the production of labelled nucleic acids for diagnostic purposes.

Pharmacia's Ready-To-Go **DNA labelling kit** allows premixed, individual reactions which are stable at room temperature (*Reader Service No. 111*). Predispensed reaction mixes are treated by a special process which turns each one into a glass, said to be stable indefinitely at ambient temperatures. Each vitrified reaction mix rehydrates when mixed with water or aqueous buffer. DNA



Ready-To-Go DNA labelling kit.

can be labelled by adding it (and labelled dCTP) to one of the premixed oligolabelling reactions provided in the kit. The use of premixed and predispensed reaction mixes is designed to eliminate the possibility of pipetting errors. And, because each reaction is premixed in its own tube, the possibility of contamination from a dirty pipette tip should be minimized.

Novex's colloidal Coomassie staining **kit for the detection of electrophoresed proteins** can now be obtained from R & D Systems (*Reader Service No. 112*). The procedure requires only one change of solution and is said to yield results in about an hour.

Equipment

Wallac has extended its range of products for digital spectral analysis with the introduction of the 1415 **benchtop liquid scintillation counter** designed for the detection of extremely low levels of α/β radiation in the environment (*Reader Service No. 113*). This

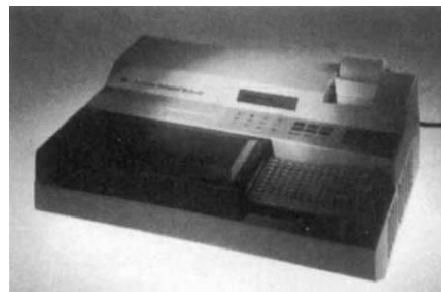


Wallac's benchtop counter for the detection of low levels of α/β radiation.

model has an 'active' guard detector that is optically isolated from the sample, so that the origin of a sample event is positively identified. Spectra can be saved on hard disk in ASCII format. Users can also access spectra through their own software programs. Ready-to-use cocktails are available that allow α/β separation without additional preparation stages.

The Fluorolog- $\tau 2$ from Instruments S.A. can be purchased as a complete turnkey, lifetime constant-wave (CW) **spectrofluorometer**, or as an upgrade to an existing Fluorolog-2 (*Reader Service No. 114*). The instrument consists of an integrated optical module and electronics rack that includes everything necessary for steady-state and frequency domain fluorescence. The company says that lifetimes in the range of 10 picoseconds to 2 microseconds with a resolution of ± 5 picoseconds can be measured using the 450 W xenon arc lamp source. A laser port is provided for when extra sensitivity is required. All of the standard CW Fluorolog-2 specifications are preserved and the system can be rapidly switched between CW and lifetime. Prices start at UK£60,000.

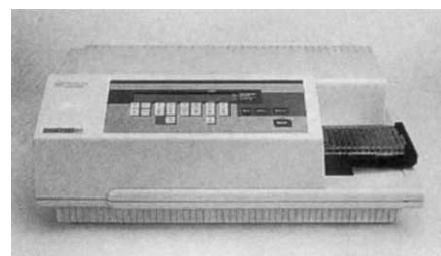
Labsystems has introduced a new range of Luminoskan **microplate luminometers** for use in nonisotopic luminescent assay



Luminoskan range of microplate luminometers from Labsystems.

systems (*Reader Service No. 115*). Three models are available ranging from the basic Luminoskan EL to the fully specified Luminoskan RS. All units can be used as part of an automated system configured with robots and controlled through a computer. The Luminoskan EL is designed for use with the enhanced luminescence or 'glow' reagents. This basic model of microplate luminometer comes without injectors, temperature control or mixing but offers a choice of eight measurement modes and computer controls. The Luminoskan RT, on the other hand, is supplied with two injectors and an orbital mixer as standard, and is said to be more suited to applications that require the addition of reagents but where there is no need for temperature control (for example, ATP studies and assays involving flash reagents such as acridinium esters). The top-of-the-line model, the Luminoskan RS, offers bi-level temperature control over the range of 20 °C to 43 °C, orbital mixing and two injectors (with the option of adding two additional injectors, if required).

SPECTRAmax 250 is the new **microplate spectrophotometer** from Molecular Devices (*Reader Service No. 116*). The instrument has a xenon flash lamp and grating



SPECTRAmax 250.

monochromator which allow direct ultraviolet measurement of DNA, RNA and protein in the microplate. Using disposable or quartz microplates, up to 96 assays can be run at one time. DNA purity can be measured with A_{260}/A_{280} ratios. In addition, spectral scans from 250-750 nm can be performed. Accompanying SOFTmax PRO microplate data analysis software provides built-in spreadsheet capabilities for data analysis and reporting.

Custom fabricated **radioisotope shipping containers** that are made from de-



Custom containers for radioisotopes.

pleted uranium, a material said to be denser and more efficient at shielding than conventional lead, are now available from Nuclear Metals (*Reader Service No. 117*). Reusable to minimize packaging costs and eliminate disposal problems, they are said to be capable of handling more material than a lead container of the same weight. Applications include the transportation and storage of spent nuclear fuel, radioisotopes and radiopharmaceuticals.

Boehringer Mannheim now offers **streptavidin matrix in prefilled columns** that are equipped with luer-lock adaptors for attachment to either a syringe or to peristaltic pump via a plastic tube (*Reader Service No. 118*). The company also offers **streptavidin-coated membranes**. These noncharged membranes can be used in many assays for the semi-quantitative detection of biotinylated peptides, antibodies, nucleotides, glycoconjugates and other metabolites. Stated applications include protein kinase/phosphatase assays, protease assays, epitope mapping, glycan analysis and the detection of any biotin-labelled compound. Also available are streptavidin-coated microtitre plates for fluorescent and luminescent analysis.

Tools for immunology

Biogenesis has a new line of **radioimmunoassay kits specifically for diabetes research** (*Reader Service No. 119*). Said to be the most significant in the range is an insulin-specific assay that does not cross-react with intact proinsulin or its predominant circulating split form, Des (31,32) proinsulin. A glucagon assay uses an assay that reacts with pancreatic glucagon, having a reported cross-reaction with enteric glucagon of less than 0.1 per cent. Also offered are kits for rat and porcine insulin and C-peptide.

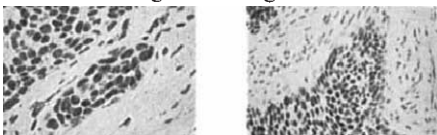
Nanoprobes has introduced two new **silver developers** for immunogold enhancement (*Reader Service No. 120*). LI Silver is light insensitive (may be viewed while developing) and is intended for blots and light microscopy. HQ Silver, however, is a high-quality silver enhancer intended for electron microscopy.



Silver developers.

It is designed to provide improved uniformity of development (more regular grains), with fewer artefacts and better ultrastructural preservation as its pH is neutral (unlike most silver enhancers which tend to be acidic).

ER/PR detection kits from BioGenex comprise a complete **immunostaining system for hormone receptor analysis** in routinely-fixed tissues (*Reader Service No. 121*). The kits are designed for rapid oestrogen receptor and progesterone receptor (ER/PR) staining. The Super Sensitive ER/PR kit for paraffin sections contains all the reagents necessary to stain 30 slides each of ER and PR, with all primary antibodies and detection reagents — link, label, substrate, chromogen and negative control —



ER (left) and PR immunostaining.

optimized and ready to use. The concentrated ER/PR antibody kit, on the other hand, contains enough concentrated ER and PR antibodies to run 200 slides of each. Run times for both can be completed in under a day, and both kits include the new nonhazardous Antigen Retrieval Citra solution, designed to enhance the staining of fixation-sensitive markers like ER and PR. The Super Sensitive kits are available with a choice of chromogen systems.

Books/catalogues

Finland-based Wallac Oy's new commissioned textbook, entitled *Bioanalytical Applications of Labelling Technologies*, provides a review of current trends and opportunities in **biospecific assays using labels** — radioactive and otherwise (*Reader Service No. 122*). Prominence is given in this 399-page hardback volume to the development of time-resolved fluorometry. This and other measurement technologies are explained with an emphasis on their most important



Labelling background for biospecific assays — see Wallac.

areas of application. There are chapters on immunoassays, DNA hybridization assays, enzyme analysis, receptor binding assays, cellular examinations, *in vivo* experiments, image analysis and environmental and archaeological analysis with low-level radioactivity monitoring. Price is about UK£25.

Now out — the 1994 **catalogue of stable isotopes, deuterated compounds and solvents** for nuclear magnetic resonance from Dr Glaser AG Basel (*Reader Service No. 123*). The catalogue lists isotopically labelled compounds, as well as noble gas isotopes and miscellaneous isotopes. □

These notes are compiled by Diane Gershon from information provided by the manufacturer. For more details, fill in the reader service card bound inside the journal. Prices quoted are sometimes nominal and apply only within the country indicated. The PCR process is covered by patents owned by Hoffman-La Roche.

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